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PROCEEDINGS OF THE TWENTY-SECOND ANNUAL MEETING OF THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION HELD IN ATLANTIC CITY, N. J., MAY 5, 1930

Liver Fractions in Pernicious Anemia. By RANDOLPH WEST and (by invitation) MARION HOWE, New York, N. Y.

It has been shown that the material in liver active in pernicious anemia (activity being measured by reticulocyte response after feeding) is not precipitated by sodium sulphate nor by alkaline silver solutions, is precipitated by phosphotungstic acid, is not precipitated by picric acid and is not removed from the water phase on shaking as picrate against butyl alcohol and ether mixtures.

During the past year we have dissolved the Lilly Liver Extract in water, saturated with solid anhydrous sodium sulphate, filtered and removed sodium sulphate from filtrate by adding alcohol to 60 or 70 per cent by volume. After blowing off alcohol an excess of solid picric acid is added, any precipitate discarded, and the filtrate shaken five times against a mixture of equal parts of ether and butyl alcohol. The water layer after shaking out butyl alcohol with ether and blowing off ether is precipitated in 3 per cent sulphuric acid with phosphotungstic acid and the precipitate regenerated after dissolving in 75 per cent acetone with hot baryta. This material is adjusted to pH 5.0 and evaporated in vacuo to 10 cc. (material from 400 grams Lilly Extract). This is poured into 190 cc. absolute alcohol. The precipitate has been fed in doses of 500 mgm. daily with practically no response. The supernatant fluid is active. On adding 10 cc. of hot aqueous baryta to the supernatant fluid an amorphous precipitate forms which is centrifuged down, dissolved in water and largely freed of barium with sulphuric acid. This material is highly active, while the final alcoholic supernatant fluid is almost inert. In one instance reticulocytes rose from 4 to 21 per cent with red cells at 900,000 on 500 mgm. daily for four days by mouth.

On hydrolysis with hydrochloric acid a crystalline hydrochloride separates which has the crystal form and empirical formula on combustion of hypoxanthine. Ozonones melting at 156° and 204°C. have been obtained.

On feeding a crystalline phosphotungstic regenerated precipitate nearly all activity was lost, though the Molisch reaction and hypoxanthine were present. Similarly there is marked impairment of activity on feeding the precipitate from ammoniacal lead regenerated with H_2S . Adenosine and the sodium salt of inosinic acid from muscle have been fed without any response. Yeast nucleic acid in 5-gram doses daily has proven inert.

The filtrate after precipitation with lead and ammonia was next used, 680 mgm. obtained from 1200 grams of the Lilly extract were injected intravenously, the

reticulocytes rising from 3 to 40 per cent with red cells rising from 1.4 to 2.3 millions (reticulocyte peak 640,000).

This material is amorphous and probably impure. It is acid to methyl red, is levorotatory (about -15). On combustion C 46.6 per cent, H 6.9 per cent, N 10.6 per cent, O 35.8 per cent; no S nor P; $\text{NH}_4\text{-N}$ before hydrolysis trace, after hydrolysis about 50 per cent of N. Amorphous Ba and green Cu salts have been obtained, soluble in water, precipitable from alcohol. The precipitate is heavy with phosphotungstic acid, crystalline, moderate with HgSO_4 , and alcoholic PtCl_4 , slight with ammoniacal AgNO_3 . There is none with picric, falcianic nor picrolonic acids, nor with AuCl_3 . Amorphous precipitate from alcohol with thalious hydroxide. No reduction KMnO_4 in acid, slight in alkaline solution. Diazo and Molisch reactions are negative. On distillation with soda lime vapors given a strong pine splinter reaction. On adding β naphthol and 2 volumes of concentrated H_2SO_4 a strong green fluorescence develops. A careful search of hydrolytic products has failed to reveal thymene, uracil or cytosine; but a substance giving a green copper salt, and precipitable by phosphotungstic acid is still present.

The best fraction is rich in a nitrogenous body with acid properties.

Quantitative Studies of the Number of Cells in the Bone Marrow. By RAPHAEL ISAACS, Ann Arbor, Mich.

The cells of the fresh bone marrow can be counted with a fair degree of accuracy by dissolving a measured amount of marrow in blood serum and then counting the cells by the usual blood counting technic. Differential counts can be made from films made from the serum suspension. The cells, under these conditions, stain the same as do those of the blood stream and can be identified easily. Data on the cell numbers and types in the normal and various diseases have been correlated. In nephritis there is a reduction in the number of adult red blood cells and polymorphonuclear neutrophilic leucocytes in the bone marrow and an increase in the number of "lymphoid" cells.

Further Observations on the Etiological Relationship of Achylia Gastrica to Pernicious Anemia. By WILLIAM B. CASTLE, and (by invitation) WILMOT C. TOWNSEND, and CLARK W. HEATH, Boston, Mass.

Observations have already been reported¹ demonstrating that by an interaction of normal human fasting gastric contents and beef muscle, both of which are separately ineffective, a substance is produced which will cause a prompt hematopoietic effect and clinical improvement in Addisonian pernicious anemia comparable to that obtained with liver feeding. The nature of this reaction has now been studied by means of observations upon fifteen additional cases of pernicious

¹ Castle, W. B.: Proc. Roy. Soc. Med., 1929, xxii, 58.

Idem: Am. J. Med. Sci., 1929, clxxviii, 748.

Castle, W. B., and Townsend, W. C.: Am. J. Med. Sci., 1929, clxxviii, 764.

anemia in respect to the nature of (a) the extrinsic factor of the reaction contained in beef muscle and (b) the intrinsic factor present in normal human gastric contents. These observations demonstrate that the proteins precipitated at pH 6 from beef muscle may act as the extrinsic factor. Normal human saliva, and duodenal contents collected by a special technique so as to be free from gastric juice are entirely ineffective when incubated with beef muscle. The essential reaction between gastric juice and beef muscle may take place in neutral solution. Heating the gastric juice to 40°C. for three days or to from 70° to 80°C. for one-half hour abolishes its effectiveness. If normal gastric juice is treated with casein solution² and then with magnesium carbonate,³ the pepsin and rennin may be completely removed, leaving behind an unknown factor which, however, can react in neutral solution with beef muscle to produce the hematopoietic substance.

In contrast to our original negative experiments with pig stomach mucosa after autolysis in acid,⁴ it has now been found possible to reproduce the activity of 300 grams of fresh pig stomach mucosa by incubating 25 or 100 grams of the fresh stomach mucosa with 200 grams of beef muscle. These experiments suggest that the effectiveness of pig stomach feeding demonstrated by Sturgis and his associates after our original negative results with autolyzed mucosa,⁵ is the result of postmortem changes within the tissue similar to physiological reactions occurring between normal human gastric juice and beef muscle. The activity of the pig stomach is furthermore apparently not identical with that of liver since heating the former to 100°C. for five minutes is sufficient to markedly effect its potency in contrast to the lack of effect on liver extract under similar circumstances.

The gastric juice of two patients with blood pictures entirely consistent with the diagnosis of pernicious anemia (sprue and multiple intestinal anastomosis), both of which subsequently showed a typical response to effective liver extracts, has been found in each case to contain normal amounts of hydrochloric acid, pepsin and rennin, but to be entirely incapable of reacting with beef muscle. The irrelevance of any of the existing tests of gastric function for the detection of the essential factor of the normal gastric juice is thus made clear.

The Response Obtained in Healthy Pigeons to the Administration of Substances Effective in Pernicious Anemia. By JANET M. VAUGHAN and GULLI LINDH MULLER (by invitation) and GEORGE R. MINOT, Boston, Mass.

Recent work on the treatment of anemia has facilitated the differentiation of a group of human anemias which must be regarded as due to the deficiency of some

² Hammarsten, O.: Ztschr. f. physiol. Chem., 1911, lxxiv, 142.

³ Idem: Ztschr. f. physiol. Chem., 1908, lvi, 18.

⁴ Castle, W. B., and Locke, E. A.: J. Clin. Invest., 1928, vi, 2.

⁵ Sturgis, C. C., and Isaacs, R.: J. Am. Med. Assoc., 1929, xciii, 747.

substance essential for the normal production of adult red blood cells. Such anemias, including pernicious anemia, are characterized by a megaloblastic hyperplasia of the bone marrow. The bone marrow of the healthy grain-fed pigeon is also of this type. It appeared therefore possible that the administration to pigeons of substances capable of alleviating a deficiency in man associated with megaloblastic hyperplasia of the marrow might provoke a response in the bird similar to that resulting in clinical cases. If such were the case it might provide a biological test for the potency of these substances.

Intravenous administration of a series of relatively pure liver preparation¹ known to be effective when given intravenously in pernicious anemia gave a consistent response in fourteen healthy birds. Two pigeons only failed to react to a potent fraction. These two negative results were obtained probably, because it was only possible to give an extremely small dose owing to scarcity of material. The response was characterized by a rapid increase in the circulating reticulocytes, from an average control level of 9.2 per cent to one of about 18 per cent. This was accompanied by a pronounced gain in the birds' weight when less pure preparations were used. Similar effects were obtained when liver extract No. 343 (N.N.R.) was given by mouth, except when sepsis was present, which is in agreement with clinical observations. No such definite responses were obtained when extracts ineffective in pernicious anemia were employed. Negative results were also obtained with intravenous injections of sodium chloride and histamine, preparations of both fractions of Vitamin B (Vitamin B¹ (G), B² (F), and liver extract No. (N.N.R.)), in which the active principle had been destroyed.

Beef muscle previously digested by healthy gastric juice is as effective as liver in pernicious anemia. Human gastric juice fed pigeons caused no reticulocyte response, but beef muscle promptly did so. Probably the pigeon's gastric juice, like normal human gastric juice, can elaborate from beef muscle material that promotes blood regeneration.

The positive action of those substances effective in pernicious anemia upon the bone marrow of the healthy corn-fed pigeon would appear to be of biological significance.

The Use of Blood Bilirubin Curves as a Test for Liver Function. By GEORGE A. HARROP, JR., and (by invitation) E. S. G. BERRON, Baltimore, Md.

The measurement of the rate of disappearance of bilirubin from the blood after intravenous injection has been employed as a method for the determination of liver function. The use of bilirubin, which is a physiological material normally excreted by the liver has obvious advantages over dyes and other foreign substances which have been employed for this purpose. In the past the practical

¹ We are greatly indebted to Dr. Edwin J. Cohn and Dr. Thomas L. McMeekin of the Department of Physical Chemistry of the Harvard Medical School who made the liver preparations and supplied them for our experiments.

difficulties of dissolving bilirubin so that it might be injected, except in extremely alkaline solution, have prevented its use. A method has now been found for dissolving the material and injecting it at the hydrogen ion concentration of the blood.

When 1 mgm. of the material per kilogram of body weight is injected, it is found that the material disappears normally from the blood stream promptly, but that with persons with liver damage this excretion is greatly delayed. Comparison with various other methods for liver function—dye injection, levulose, etc., shows that it is very much more delicate. By this method it has been possible to demonstrate hepatic disturbances particularly in chronic long standing anemia, in mildly decompensated cardiac disease and in acute infections, particularly lobar pneumonia, which were hitherto not suspected.

Electrolyte Studies in Histamine Shock. By DANA W. ATCHLEY, and (by invitation) DICKINSON RICHARDS and E. N. BENEDICT, New York, N. Y.

Histamine shock was studied in a series of dogs using blood samples drawn before, during and after the effect of the drug. Consistent results were observed as follows:

1. Extraordinary increase in hematocrit with no change in serum protein present; indicating removal of whole serum.
2. Striking increase in undertermined acids (B-A); proved by analysis to be lactic acid.
3. Lactic acid acidosis compensated by (a) increase in total base, (b) decrease in bicarbonate.

The unique response to acidosis by increase of base has been noted by L. J. Henderson and A. Bock in the lactic acid acidosis of prolonged exercise.

The Participation of an Unidentified Tissue Substance in the Formation of Wheals. By H. L. ALEXANDER, St. Louis, Mo.

It has been shown¹ that if a constant amount of histamine be injected intradermally at different sites, the resulting wheals after a given time will vary greatly in size. The same variation occurs with morphine,² allergens,³ etc. In general, larger wheals appear on the trunk than on the extremities. Such wheals are presumably due to the release of a histamine-like substance (H-substance)⁴ by the skin cells at the injected sites. This, in turn, acts on the surrounding capillaries, making them permeable to their contained plasma. This theory, probably correct, does not account for the inconstant size of wheals at different sites. There is no evidence that this is due to differences in skin structure or vascularity. It occurs in dogs as well as in human subjects.

¹ Lewis, T., and Grant, R. R.: Heart, 1924, xi, 209.

² Gröbel, A.: Zeit. exp. Med., 1929, lxx, 352.

³ Alexander, H. L.: Proc. Soc. Exp. Biol. and Med., 1928, xxv, 800.

⁴ Lewis, Thos.: The Blood Vessels of the Human Skin and their Responses, London, 1927, Chap. VI.

On the assumption that some tissue factor other than H-substance contributes to wheal formation, the following experiment was done. Skin removed from an anaesthetized dog was washed, chopped and shaken for two hours in nine parts of 0.9 per cent sodium chloride solution (= skin extract). A histamine phosphate solution 1 to 10,000 also in sodium chloride was prepared (= histamine). Of each of the following 0.02 cc. were injected intradermally in a second dog: (a) histamine; (b) skin extract; (c) skin extract + histamine (histamine added to concentration of (a)). After fifteen minutes, the increase in size of wheal formation was measured with a planimeter. The average readings of a series were (a) 0.3 sq. cm., (b) 0.0 sq. cm., (c) 0.8 sq. cm. The increase in (c) was constant in several hundred injections. Atropine and morphine reacted similarly with skin extract. Extract of human skin in fewer experiments likewise enhanced the action of histamine.

It is presumed that there is a substance in the skin, irregularly distributed, that enhances the action of wheal forming substances either directly or through the mediation of H-substance. Chemical analysis shows this to be non-dialyzable. Its identification is not completed.

Changes in the Tissues Following the Administration of Excessive Quantities of Water. By C. H. GREENE and L. G. ROWNTREE, Rochester, Minn.

Several cases of diabetes insipidus have been observed in which the excessive intake of fluids was accompanied by restlessness, agitation, muscular tremor and the development of epileptiform convulsions. Rowntree has described the experimental production of a similar condition in animals, under the name of water intoxication. We have previously demonstrated that the administration of excessive quantities of water will produce a true dilution of the blood.

The accompanying changes in the tissues were studied in a series of young male white rats of approximately 200 grams body weight. Distilled water, in quantities of 5 per cent of the body weight was administered by mouth or intraperitoneally at half-hourly intervals until convulsions occurred. The rats were then killed by bleeding and determination made of the gross and net weight of the animal, of the weight of the eviscerated carcass comprising the skin, skeleton and musculature and of the weight of the brain, liver, kidney, spleen and remaining viscera. The per cent of total solids in the blood, muscles and other organs was also determined.

In general the changes observed were the same whether the water was given by mouth or intraperitoneally. Comparison of the two experimental series of rats with the normal control series shows that there was an increase in weight of between 25.0 and 27.0 grams on the average, or between 12 and 13 per cent of the total body weight. The changes in the blood were similar to those previously reported.

The increase in the trunk was responsible for the greater portion of the increase in weight. A reduction, in the total solid content in the muscle, on the average from 24.28 to 19.65 and 20.97 per cent respectively suggests that the greater part of the water absorbed was taken up by the muscles. There was a slight increase in

the weight of the brain with an accompanying decrease in the per cent of solids in that organ. These changes were the most striking. The individual variation in the size of the liver, kidney and spleen was so great as to mask any gross changes in these organs, though there was a slight reduction in the percentage of solids in each case.

Studies in Edema. I. The Mechanism of Water Diuresis in Man. By FRANK FREMONT-SMITH, and (by invitation) MAURICE FREMONT-SMITH, MARY ELIZABETH DAILEY, PHILIP SOLOMON, DEWITT STETTEN, Jr., and MARGARET P. CARROLL, Boston, Mass.

By parallel determinations of creatinine in serum and in urine, it is possible to calculate the amount of glomerular filtrate formed per hour (Rehberg, P. B., *Biochem. J.*, 1926, xx, 447). This we have done during a standard water diuresis test (200 cc. of water every half hour for three hours), both in normal individuals and in patients with various kinds of edema. In addition we have studied the effects of pituitrin, caffeine and urea. Specific gravity, freezing-point depression, creatinine, urea, and chloride have been determined in serial samples of urine, while freezing-point depression, creatinine, urea, chloride, protein, and total solids have been determined in the blood serum.

The normal response to water drinking is a prompt diuresis exceeding in volume the water intake. Little, if any, dilution of the blood serum occurs. In the presence of edema, at the onset of fever, or after pituitrin injection, however, this normal diuresis does not occur, or is delayed, while a significant dilution occurs in the blood serum.

At the height of water diuresis, when the hourly volume of urine is 700 cc. or more, the amount of glomerular filtrate formed may not be appreciably greater than when the urine excreted is only 30 cc. per hour.

Vimtrup (*Am. J. Anat.*, 1928, xli, 123) has shown that the average glomerulus in man contains fifty non-anastomosing capillaries, while Richards and Schmidt (*Am. J. Physiol.*, 1924, lxxi, 178) have shown in the frog that variations occur not only in the number of glomeruli functioning at any one time but also in the number of patent capillaries in a given glomerulus. On the basis of the above known anatomy and physiology of the glomerular capillaries, we would emphasize the importance of the distribution of the glomerular capillary bed for the control of tubular reabsorption. Thus it is possible to have the same amount of glomerular filtrate formed from one glomerulus with fifty open capillaries as would be formed in the same time from fifty glomeruli with only one open capillary in each. In the first case, however, all the glomerular filtrate would pass rapidly down one tubule; in the second case the same volume of filtrate would be distributed into fifty tubules each receiving one fiftieth of the total, and the flow down each tubule would be proportionately slower. Cushny (*J. Physiol.*, 1902, xxviii, 431) and others have presented evidence that the slower the rate of flow of glomerular filtrate down the tubule the more will it be concentrated. Hence a small volume of concentrated

urine would be the result if the filtrate is formed in a large number of glomeruli with but one capillary open in each, while a dilute urine of large volume may be expected if the filtrate is formed in relatively few glomeruli in each of which all or most of the capillaries are open, for example, at the height of water diuresis. Thus a redistribution of a given volume of blood flow from many glomeruli with few capillaries open, to few glomeruli with many capillaries open, could produce the extreme changes in the urine volume and concentration occurring during water diuresis, when only minor changes in the amount of glomerular filtrate occur. A similar redistribution would explain the results of Pickford and Verney (Am. J. Physiol., 1929, xc, 470) who found in a heart-lung preparation that the ligation of a primary arterial branch to the kidney did not produce a reduction in urine volume proportional to the diminution in blood flow. In fact, not infrequently, an absolute increase in urine flow occurred at constant perfusion pressure.

Other conditions being constant, the amount of glomerular filtrate formed in a given time is a linear function of the number of glomerular capillaries patent no matter what their distribution, while the extent of reabsorption in the tubules at any given time is a function of the number of tubules active.

Variations in the distribution of the glomerular capillary bed allows wide range in the number of tubules available for the concentration of any given amount of glomerular filtrate. All the filtrate derived from fifty capillaries may be poured into one tubule or distributed into fifty tubules.

This offers a differential mechanism for the excretion of water and of solids, which, if substantiated, must play an important rôle in the regulation of both the volume and the composition of the organism. The two contrasting clinical conditions, uremia without edema, and edema without nitrogen retention, now become understandable on the basis of functional or structural changes in the distribution of the glomerular capillary bed. On the one hand, the glomerular filtrate is deficient in quantity, but so distributed into relatively few tubules (from glomeruli with many capillaries open in each) that there is little reabsorption. A polyuria of low specific gravity results; edema is absent. The small contracted kidney and the polycystic kidney are examples. On the other hand, as in nephrosis, the glomerular filtrate is normal in quantity, but is always distributed into so many tubules (from glomeruli with but few capillaries open in each) that much reabsorption takes place leading to edema. A small volume of concentrated urine is excreted and water diuresis fails to occur. When both the glomerular filtration surface is deficient and the available glomeruli have but few patent capillaries in each, both nitrogen retention and edema take place.

At autopsy the glomerular pathology is suggestively in accord with this conception (Bell, E. T., Am. J. Path., 1929, v, 587).

The Relation of Edema and of Fatigue to the Potassium Content of Skeletal and Cardiac Muscle. By TINSLEY RANDOLPH HARRISON and (by invitation) J. ALFRED CALHOUN, GLENN E. CULLEN, and COBB PILCHER, Nashville, Tenn.

Previous studies have shown that the cardiac and skeletal muscles of individuals

dying of congestive cardiac failure contain an abnormally small amount of potassium. In the present study an attempt has been made to determine the reasons for this phenomenon.

Edema appears to be the cause of the loss of the potassium from skeletal muscle because:

1. The skeletal muscle of individuals with congestive heart failure has an abnormally high water content.
2. Diminished potassium content of skeletal muscle is found in subjects with edema due to other causes.
3. The potassium content of skeletal muscle of patients who have cardiac disease but who have never had edema is normal.
4. The potassium content of skeletal muscle rises as the patient loses edema.
5. The daily injection of saline solution, or Ringers solution, into one of the hind legs of a dog results after a week or more in a marked decrease in the potassium content of the "edematous" leg—the normal leg showing no such diminution.

These changes are not simply dilution effects as the potassium content of "dry" as well as "wet" muscle is affected.

Edema does not appear to be the cause of the loss of potassium from the cardiac muscle as the water content of the hearts of patients dying of congestive heart failure is usually normal or nearly normal.

The loss of potassium from the cardiac muscle appears to be related to "cardiac fatigue" for the following reasons:

1. In patients with hypertension, but without congestive heart failure the potassium content of the right ventricle has been found to be normal; that of the left ventricle low.
2. In a patient dying of massive collapse of the lung, the potassium content of the left ventricle was normal, whereas, that of the right ventricle was diminished.
3. In a patient with *concretio cordis* with the peripheral signs of cardiac insufficiency, but with a small atrophic myocardium, the potassium content of both ventricles was higher than normal.
4. Stimulation of the sciatic nerve for several hours results in a diminished potassium content of the muscles of the stimulated leg as compared to the "control" normal leg.
5. Tachycardia resulting from vagotomy may cause diminution in the cardiac potassium.
6. Ligation of one of the primary branches of the pulmonary artery causes a change in the potassium content of the right ventricle but no change in that of the left ventricle.
7. Digitalis causes a change in the potassium content of the heart.

It is believed that the loss of potassium brought about by edema and by overwork is in each instance, related to a state of relative tissue oxygen lack.

Potassium dibasic phosphate was administered to a group of patients with congestive cardiac failure. In those of this group who came to autopsy the skeletal

muscle contained normal amounts of potassium but the cardiac muscle contained only slightly more than that of a similar group who had not received this salt.

A study of the relationship of potassium loss to other electrolyte changes is in progress.

Studies on the Experimental Transmission of Acute Upper Respiratory Infection. (Common Cold). By GERALD S. SHIBLEY, A. R. DOCHEZ, and (by invitation) KATHERINE C. MILLS, New York, N. Y.

Further progress in the experimental transmission of human colds to apes by means of filtrates is presented. In addition, a limited number of transmission experiments in which human subjects were used is reported.

To the present, we have successfully passed colds, by intranasal inoculation with filtered nasal washings, from humans to apes in 44 per cent of the cases attempted. Further, we have transmitted colds from ape to ape by the same method. Attempts to transmit colds have been unsuccessful when we have used filtrates which have been kept from 10 days to 2 weeks and when we have used for purposes of inoculation the Gram-negative filter-passing organisms (described by Olitsky and his co-workers) obtained from patients suffering from colds.

In the human experiments the subjects have been taken singly and have been placed in rigid quarantine. To rule out possible entrance during incubation of colds, and for purposes of preliminary bacteriological study, the subjects have been kept under observation from 5 to 7 days. At the end of this period, filtered nasal washings, obtained from individuals suffering from typical colds, have been inoculated intranasally. To the present, we have been successful in transmitting colds in 43 per cent of the cases attempted.

Variations in the Nasopharyngeal Flora in the Tropics (Virgin Islands, West Indies) during the Period of a Year. By W. G. SMILLIE, Boston, and (by invitation) FRANK MILAM, New York.

The report is upon part of a study of the influence of environmental factors upon variation in the nasopharyngeal flora. A field research laboratory was established in St. John's, U. S. Virgin Islands, or a small, isolated island in the West Indies. Epidemiological, bacteriological, and climatological observations were continued for one year.

The basic nasopharyngeal flora were found to be similar to the types observed in southern Alabama and Labrador. Some interesting and striking differences were noted. One mild epidemic of colds occurred and was studied. The correlation of the occurrence of upper respiration infections with variations in atmospheric temperature was noted.

The Spread of Rheumatic Fever through Families. By JOHN R. PAUL and (by invitation) ROBERT SALLINGER, New Haven, Conn.

A familial study upon rheumatic fever has been engaged upon in the belief that it might bring us into closer contact with the hereditary and environmental condi-

tions under which this obscure disease naturally develops, than it is possible to establish through observations upon isolated hospitalized patients.

To attack the problem we studied twelve families. Selection was made on the following bases: (1) that at least two or more members of the family shall have suffered from rheumatic fever, and (2), that all of the members of the family shall have been under observation at the New Haven Hospital or Dispensary for a reasonable period of years.

The method of recording data is that devised by Opie in his studies on the spread of tuberculosis through families.

The character of the distribution of rheumatic fever through these families strongly suggests that we are dealing with a chronic disease which is moderately infectious. The appearance of an active flare-up of rheumatic fever in one member of the family has been frequently accompanied by simultaneous outbursts of the disease in other members and not infrequently by the appearance of non-specific types of illness, such as infectious arthritis, bronchitis, bronchopneumonia unexplained skin rashes, etc.

The spread of the disease occurs more frequently to the younger rather than to the older members of the family.

Serological Reactions in Pneumonia with a Non-protein Somatic Fraction of Pneumococcus. By WILLIAM S. TILLET and (by invitation) THOMAS FRANCIS, JR., New York, N. Y.

Up to the present time the two chemical constituents of pneumococcus cells employed in immunological and serological reactions have been:

1. Soluble specific substance (Type-specific carbohydrate).
2. Somatic protein (so-called nucleo-protein).

The present report is based upon observations made with a third chemical fraction derived from pneumococci and chemically distinct from the other two (designated Fraction "C"). The chemical nature of Fraction "C" has been determined by Dr. Walther F. Goebel and is reported in a separate communication. At the present time it is sufficient to state that it is non-protein and appears to be a carbohydrate common to the pneumococcus species.

Sera obtained at frequent intervals from patients acutely ill with, or convalescent from pneumonia have been mixed with varying dilutions of Fraction "C" and the presence or absence of precipitation noted. It has been found that serum derived from a patient during the acute phase possesses a high titre of precipitins for Fraction "C." Within a day or two after recovery this precipitating power abruptly and permanently disappears. The sera of 50 patients have been tested at frequent intervals from admission to the hospital until several months after recovery. In every instance the precipitating capacity for Fraction "C" has run a similar course. The phenomenon is unrelated to the type of pneumococcus causing infection.

The curve of the precipitin titre for Fraction "C" is distinctly different from that obtained by the use of either type-specific carbohydrate or nucleo-protein fractions.

Type-specific antibodies are absent during the acute phase of pneumonia, are demonstrable at about the time of crisis, and are homologous to the infecting organism. On the other hand, anti-"C" antibodies are highest during the acute stage, disappear just after crisis, and are not related to type specificity. Anti-protein antibodies do not vary markedly during the course of pneumonia. Consequently it is possible to plot three distinct curves of antibody titre depending upon which of three chemically distinct fractions of pneumococcus is used as precipitinogen.

Sera derived from patients suffering from febrile diseases other than pneumonia have also been tested for the capacity to react with Fraction "C." Of the limited number so far investigated, precipitins of Fraction "C" have been found only in those individuals acutely ill with diseases, whose etiological agent is known to be, or suspected of being a Gram-positive coccus. In those instances anti-"C" antibodies are demonstrable during the active febrile states and are not present during periods of normal temperature.

Bacteriological Studies on Rheumatic Fever. By RUSSELL L. CECIL, and (by invitation) EDITH E. NICHOLLS, and WENDELL J. STAINSBY, New York, N. Y.

Streptococci have been obtained in a high percentage of patients with rheumatic fever, both from the blood and from the affected joints. Most of these streptococci have been classified as alpha streptococcus (*Streptococcus viridans*). Occasionally, however, beta or gamma streptococci are isolated. Agglutination and absorption tests indicate that a considerable number of the strains of streptococci recovered from the blood and joints of patients with rheumatic fever show a tendency to fall into definite biological groups. The serum of patients with rheumatic fever usually shows agglutination for the type of streptococcus recovered from the blood and joints. These findings corroborate those of previous investigators and tend to establish the conclusion that rheumatic fever is a streptococcal infection, usually of the alpha or *viridans* type. The pathogenesis of rheumatic fever in respect to the joint lesions appears to be analogous to that of infectious arthritis and gonococcal arthritis. Bacterial allergy probably influences the clinical picture in all three conditions, but in each instance the joint manifestations are primarily dependent upon localization of bacteria in the joint with subsequent infection.

Experimental Acute Glomerulitis. By FRANCIS D. W. LUKENS (by invitation) and WARFIELD T. LONGCOPE, Baltimore, Md.

For many reasons it has seemed impossible heretofore to produce with any regularity a diffuse glomerulitis in animals by the intravenous injection of bacterial toxins or bacteria. It was therefore decided to bring these substances, in concentrated form, in more direct contact with the kidney. Filtrates from the growth of haemolytic streptococci, lysates of haemolytic streptococci and dead bodies of haemolytic streptococci were therefore injected directly into the left renal artery in rabbits. The animals were killed at different intervals after the operation. By this method it was possible to produce a unilateral inflammation of intense degree

which often involved every glomerulus, sometimes affected the interstitial tissues and usually resulted in acute degenerative lesions in the tubular epithelium.

Experimental Studies on Lung Collapse in the Rabbit. By ROBERT G. BLOCH, (by invitation) and FRANKLIN C. McLEAN, Chicago, Ill.

These experiments were undertaken to study the mechanical intrathoracic effects of lung collapse, the influence on the pleural structure, and the changes in respiration. Seventy normal rabbits were treated over periods of five minutes to eleven months.

The anatomical conditions in the human and the rabbit's chest differ so widely that experimental results can be applied to the human chest only very cautiously.

The average rabbit's lung re-expands almost completely within twenty-four hours after a good collapse with atmospheric air. The periphery expands first. The intervals between filling could gradually be lengthened up to ten days. A marked non-inflammatory thickening of the visceral pleura occurs. It is concluded that the absorption of air is directly proportional to the thickness of the pleura.

The abdominal and thoracic respiration together with the intrapleural pressure were recorded during the procedure of collapse and afterwards. Even extreme collapse does not change the abdominal respiration materially, but increases the thoracic respiration about five times and strains the uncollapsed lung to capacity. These results suggest that treatment by simultaneous bilateral pneumothorax as a clinical procedure should be looked upon with great reserve.

Sympathetic Inhibition of the Large Intestine in Hirschsprung's Disease. By W. J. MERLE SCOTT and J. J. MORTON, Rochester, N.Y.

A clinical and experimental study of sympathetic inhibition in the large bowel is presented. In two cases of megacolon the motor activity of the large bowel was tested by its ability to expel a barium enema. All nervous impulses coming from the lumbar and sacral segments of the spinal cord were then blocked off by novocaine. After the induction of this spinal anesthesia, most of the contents of the large bowel were expelled, showing a marked increase in the motor function. In one of these cases we have subsequently removed both lumbar sympathetic chains with striking improvement. Before operation this seven year old boy had never had a spontaneous bowel movement. Now his bowels move daily without assistance. It is also shown in cats that the dilated colon, the motor function of which is inhibited by sympathetic impulses (through exposure of the bowel), shows vigorous motor activity upon the induction of spinal anesthesia.

These observations demonstrate that the motor anomaly in at least one type of megacolon is dependent upon sympathetic inhibition of the large bowel. The effect of lumbar anesthesia on the motor function of the colon provides a test for this type of megacolon and furnishes a prognosis as to the effect of lumbar sympathectomy in an individual case.

Total Electrolyte Studies and Hydrogen Ion Concentration in Normal and Abnormal Pregnancy. By JOHN P. PETERS and (by invitation) DAVID M. KYDD, New Haven, Conn.

The pH as determined gasometrically on the venous serum of 10 normal pregnant women was found to lie in the same range as the pH of 8 normal persons. The reduction of bicarbonate content of about 8 volumes per cent was compensated by a fall of about 4 mm. in CO₂ tension. In eclampsia the pH was lowered at the time of the convulsion which is similar to what occurs in severe muscular exercise. Observations of pH in other toxemias were inconsistent except in cases of simple edema when they were normal.

Total base in the toxemias is not markedly altered from the normal pregnant figure except in convulsions when it is higher than the ordinary level. Total serum proteins are reduced in normal pregnancy entirely at the expense of the albumin fraction, the globulin remaining at about the normal non-pregnant level. Edematous toxemias show serum protein figures below the normal pregnant level, but in the other toxemias there is little change in the serum proteins except in cases of dehydration where, as would be expected, they are elevated.

In cases of vomiting of early pregnancy the serum chloride is not markedly altered and in the vomitus of two cases no free hydrochloric acid and equivalent amounts of base and chloride have been found.

The changes in the acid-base equilibrium appear to be related not to the types of toxemias recognized in the usual classification but to the chief presenting symptoms.

The Dynamics of the Circulation in Patients with Coarctation of the Aorta. By HERRMAN L. BLUMGART, JOHN S. LAWRENCE, and (by invitation) A. CARLTON ERNSTENE, Boston, Mass.

Although coarctation of the aorta has been diagnosed ante-mortem in almost eighty patients, no studies of the blood flow have hitherto been available. The

TABLE 1

The resting oxygen utilization in subjects with coarctation of the aorta

Subject	Date	Brachial artery			Antecubital vein			Femoral artery			Femoral vein			Arteriovenous difference	
		Oxygen content	Oxygen capacity	Percentage saturation	Oxygen content	Oxygen capacity	Percentage saturation	Oxygen content	Oxygen capacity	Percentage saturation	Oxygen content	Oxygen capacity	Percentage saturation	Arm	Leg
		volumes per cent	volumes per cent		volumes per cent	volumes per cent		volumes per cent	volumes per cent		volumes per cent	volumes per cent		volumes per cent	volumes per cent
D. W.	June 26, 1929	17.73	19.64	90.3	16.11	19.83	81.2	17.74	20.35	87.2	13.85	19.77	70.1	1.62	3.89
J. M.	Jan. 7, 1930	16.07	17.86	89.9	9.71	17.85	54.4				5.51	17.97	31.4	6.36	10.56

purpose of this communication is to present the clinical findings in two patients with coarctation of the aorta together with the oxygen capacity and content of the arterial and venous blood of the arms as compared to that of the legs, the pulse tracings of the carotid, brachial and femoral arteries, the blood pressure of the brachial, femoral and popliteal arteries, the arteriolar pressure in the upper and lower extremities, and the velocity of blood flow both above and below the coarctation of the aorta as measured by the radium active deposit method.

As in practically all cases of this kind, the arterial pressure above the coarctation was at a hypertensive level whereas the arterial blood pressure in the legs was much lower. The results of the blood gas measurements are shown in the accompanying table (table 1). These results and the measurements of the velocity of blood flow demonstrate that while the blood flow of the legs is diminished it is within normal limits. Our studies indicate that the arterial tension above the site of the coarctation is elevated to a degree sufficient to maintain an adequate blood flow in the areas below the constriction. The elevation of arterial pressure in essential hypertension may well have a similar physiologic significance.

The Order of Excitation of the Ventricles in Bundle Branch Block. By A. G. MACLEOD (by invitation), FRANK N. WILSON and PAUL S. BARKER (by invitation), Ann Arbor, Mich.

In experiments still in progress, it has been found that semi-intrinsic or intrinsicoid deflections may be obtained from the ventricles of the dog when the heart is covered by a pad of gauze, 1.5 to 2 cm. thick, soaked in normal saline solution. Compared with true intrinsic deflections, these deflections are smaller in amplitude and less steep when the recording instrument is used at the same sensitivity. Intrinsicoid deflections can also be obtained from the ventricular cavities by a blood contact. In the case of the auricles, intrinsicoid deflections cannot be obtained either through gauze of the thickness mentioned or from the auricular cavities. It is found that true intrinsic deflections from these chambers are rapidly degraded by increasing the size of the exploring electrode.

The difference between auricles and ventricles is due, so it is suggested, to the difference in the manner in which the excitation process spreads over the ventricular as compared to the auricular muscle.

When right bundle branch block is produced in dogs the intrinsicoid deflections obtained over the right ventricle are late; those obtained over the left ventricle are early. In left branch block the reverse is the case.

In man intrinsicoid deflections can be obtained by placing the exploring electrode upon the precordium. The observations carried out thus far on patients with bundle branch block indicate that in what is at present called right bundle branch block the right ventricle is activated in advance of the left thereby supporting the view

expressed by Barker, Macleod, Alexander, and Wilson¹ that the electrocardiograms at present attributed to right branch block are the result of left branch block.

Comparative Sensitivity to Oxygen-Want and to Sodium Lactate of the Hearts of Normal and Thyroxinized Animals. By E. COWLES ANDRUS, and (by invitation) DONALD MCEACHERN, WILLIAM A PERLZWEIG, and SARAH HERMAN, Baltimore, Md.

The auricles of two rabbits were suspended side by side in a Dale bath. To one animal thyroxin had been administered in graduated doses over a period of two weeks.

The authors have confirmed the observation of J. K. Lewis, that the spontaneous rhythm of the thyroxinized heart persists after isolation at a rate conspicuously more rapid than that of the normal. They have further shown:

1. That the auricles of the thyroxinized animals are far more sensitive to the withdrawal of oxygen than the normal.
2. That their amplitude of contraction is depressed by doses of sodium lactate which produce a little or no effect upon the normal auricles.
3. That recovery from the above takes place more rapidly in the normal than in the thyroxinized auricles.

The authors regard these results as indirect evidence of an accumulation of lactic acid in the heart of the thyroxinized rabbit.

More direct evidence has been obtained by determinations of the lactic acid, glucose and glycogen content of the ventricles of normal and thyroxinized rabbits. The average values are recorded below:

	Lactic acid	Glucose	Glycogen
	<i>mgm. per cent*</i>	<i>mgm. per cent*</i>	<i>mgm. per cent*</i>
Normal (7 experiments).....	34.0	164	314
Thyroxinized (7 experiments).....	59.3	188	116

* Percentage net weight.

These indicate that, in these experiments, thyroxin has brought about an accumulation of lactic acid and a depletion of the glycogen content in the myocardium.

Observations concerning Cerebral Circulation in Man. By WM. G. LENNOX and (by invitation) ERNA LEONHARDT and ADELAIDE BUIST, Boston, Mass.

In more than 100 instances we have measured the gaseous content of blood from the internal jugular vein, and compared it with that of blood drawn from other more superficial vessels. We found that though there was a wide scattering in

¹ Barker, Macleod, Alexander, and Wilson: Trans Assoc. Am. Phys., 1929, xliv, 125.

individual patients, the average measurements showed that blood leaving the brain was more reduced than blood leaving an extremity.

Calculated respiratory quotients were 0.95 for the brain, 0.82 for the arm and 0.78 for the leg.

When patients breathed pure oxygen, the increase in the oxygen saturation of the blood in the femoral vein was greater than the increase in the internal jugular vein. When a mixture of carbon dioxide and oxygen was breathed the reverse was true. The blood of the femoral vein was not changed, whereas that from the internal jugular became much more like arterial. These correspond with observations by Wolff and Lennox on the response of pial vessels of the cat to similar stimuli.

These observations suggest either a slow blood flow or high metabolic rate of the brain, or both; that the respiratory quotient of the brain is higher than that of skin and muscles; and that the intracranial vessels may react to chemical stimulus in a different manner from vessels in the extremities.

The Circulatory Mechanism and Unilateral Edema in Cerebral Hemiplegia. By SOMA WEISS and (by invitation), LAURENCE B. ELLIS, Boston, Mass.

Clinical observations frequently reveal vasomotor changes over the paralyzed side in cases of hemiplegia resulting from cerebral vascular lesions. Unilateral edema, usually localized over the upper portion of the body, is often one of the manifestations of alteration in the circulation of such patients with hemiplegia. No extensive study has been made heretofore, however, of the nature and extent of functional alterations in the circulation in the affected part of the body, and no adequate explanation has been offered of the mechanism by which this form of edema is produced.

This communication reports studies of the mechanism of the circulation and the formation of unilateral edema in patients suffering from hemiplegia. The observations indicate that the surface temperature of the body is increased over the paralyzed side of the body, particularly over the upper extremity. The difference between the oxygen content of the cubital artery and vein is always less over the paralyzed side of the body than over the normal side. A similar divergence from the normal in oxygen difference between the femoral arteries and veins is observed to a lesser degree and more infrequently. Microscopic observations of the capillaries of the fingers of the paralyzed side reveal brighter and slightly more prominent skin capillaries, in which the velocity of the blood flow is increased, as compared with the normal side. The arterial pressure of the brachial arteries and the venous pressure in the cubital veins are essentially alike on both sides. The cardiac output in hemiplegia is either normal or slightly below normal. Decrease in the basal metabolism rarely occurs. Circulatory changes may appear immediately after onset of the cerebral accident and last for years. These changes are not necessarily related to the tone of the musculature or to the degree of the paralysis. They occur in the presence both of normal and of elevated blood pressure. The most marked unilateral edema develops in patients with previous myocardial failure.

It is concluded that in cerebral hemiplegia, following disturbance of the central vasomotor regulation, a dilatation of the arteriolar system of the upper part of the body occurs. The diffuse arteriolar dilatation leads to increased blood flow and increased capillary pressure over the paralyzed side. This increased capillary pressure may lead, especially in association with poor cardiac function, to increased rate of filtration of fluids to the tissue and hence to edema. The functional state of the vessels in the affected side of patients with hemiplegia represents a burden on the circulation which, in the absence of increased cardiac output, is chiefly carried by the normal part of the body. The circulatory changes observed in cases of hemiplegia with marked unilateral edema may have a bearing on the mechanism of the circulation and the formation of edema in congestive heart failure.

A number of nervous, chemical and mechanical responses of the minute vessels of various parts of the body indicate that the vasomotor response and sensitivity of the minute vessels is greater in the upper than in the lower part of the body. This difference in the response of the vessels at various body levels explains the more marked changes in the circulation and the appearance of edema in the upper extremity. The fact that the resistance of the minute vessels increases progressively toward the feet is of special teleological and physiological significance in man, whose body is disproportionately high in relation to its size.

The Differentiation and Significance of Certain Ophthalmoscopic Pictures in Hypertensive Diseases. By ARTHUR M. FISHBERG, (by invitation) and B. S. OPPENHEIMER, New York, N. Y.

The ophthalmoscopic findings in 280 cases of hypertensive and renal diseases were analyzed in relation to the clinical picture and to the findings at necropsy in 42 of the cases. It was concluded that the traditional unitary conception of "albuminuric retinitis" really includes three pathogenetic entities, the differentiation of which is of diagnostic and therapeutic importance:

1. The arteriosclerotic retinopathy.
2. Malignant hypertensive neuro-retinitis.
3. Choked disk due to edema of the brain.

The ophthalmoscopic pictures of these types of retinal change are described and their occurrence in the individual renal and hypertensive diseases investigated.

In each of the 11 cases of essential hypertension with malignant hypertensive neuro-retinitis that came to necropsy, necrosis of the arterioles of the kidney was present. No instance of essential hypertension with necrosis of the renal arterioles was encountered in which this retinal picture had not been present. However, the same type of retinal change also occurred in glomerulo-nephritis and in a case of suprarenal tumor with hypertension.

The arteriosclerotic retinopathy was found most often in essential hypertension but was also present in chronic glomerulo-nephritis as well as in one case of amyloid contracted kidney.

The process of healing of malignant hypertensive retinitis was followed in one

Effect of Liberal Carbohydrate Diet upon the Blood Fats in Diabetes. By H. RAWLE GEVELIN, New York, N. Y.

A selected group of fifteen children carefully studied for one to five years prior to the discovery of insulin who have been on a normal diet with insulin ever since, are compared with a group who have been on more of an old fashioned type of diabetic diet and where the blood cholesterol has remained high. We contrast this group with the normal diet group as regards blood fat, cholesterol and sugar and the effect of high carbohydrate upon reducing the blood fat and blood cholesterol in serial determinations after meals and over longer periods of time. The comparative effects of high carbohydrate, low fat diets with the conventional diet upon weight and growth are noted.

The Whole Blood Iron in Normal and Anemic Individuals. By WILLIAM P. MURPHY, and (by invitation) JOHN POWERS and RALPH LYNCH, Boston, Mass.

In an effort to utilize a more accurate means of following the effect of various types of treatment on the blood of anemic patients other than those with pernicious anemia, a method has been employed for determining the whole blood iron. In addition to the use of this method in following the cases under treatment with various means such as iron in the form of ferrous carbonate, liver, or liver extract and various combinations of these substances, whole blood iron determinations were made on a group of normal individuals in order to attempt to establish a normal whole blood iron figure and a means has been suggested through these determinations of distinguishing between the blood of patients with pernicious anemia and that in patients with so-called secondary anemia.

Observations have also been made which may be made use of in determining the percentage of iron in hemoglobin and so rather accurately the actual hemoglobin content of the blood.

A Study of Inorganic Sulphates in Relation to the Acid-Base Equilibrium in Renal Insufficiency and "Renal Acidosis." By E. G. WAKEFIELD, (by invitation) and N. M. KETH, Rochester, Minn.

In this work, data have been obtained concerning the concentration of various anions and cations in the blood serum of patients who had renal insufficiency and in a few who had "renal acidosis." The fluctuation of the electrolyte content of the blood serum was less than 15 per cent of the total amount, although wide variations in the concentration of individual ions were observed. In spite of a concentration of inorganic sulphate up to 16 millimolecular equivalents, there was still an undetermined deficit of acid. The fluctuation of the total base was greater than the maximal concentration of inorganic sulphates. There was no constant relationship between concentration of chlorides, phosphates, carbonates and sulphates. Concentrations of lactic acid were low in all the determinations, indicating that the underdetermined acid is not lactic acid.

Creatinine Excretion in Abnormal States of Nutrition. By GEORGE BOOTH, H. B. McCLUGGAGE, (by invitation) and FRANK A. EVANS, Pittsburgh, Pa.

The amount of creatinine excretion in obese patients is independent of their excess weight, and proportional to their ideal weight. During dietary reduction, the creatinine excreted is not changed.

The amount of creatinine excretion in undernourished patients is less than that normal for them if of ideal weight. During dietary correction, the creatinine excretion increases. After attaining a certain level, it remains the same even if the weight continues to increase.

Diphtheria as a Cause of Late Heart Block. By STUYVESANT BUTLER, (by invitation) and SAMUEL A. LEVINE, Boston, Mass.

A study was made of all the cases at the Peter Bent Brigham Hospital and of those seen in consultation by one of us, of complete heart block that were not due to obvious conditions such as coronary thrombosis, fever, rheumatic infection, or digitalis. This limited the group to twenty cases. It was found that in one-half of these cases there was an antecedent history of diphtheria. We have felt for some time that diphtheria was in some way related to subsequent complete heart block, because not infrequently comparatively young individuals have complete heart block, where there is no good cause and where even arterio-sclerosis is absent. Such young patients have given a past history of diphtheria. It is also known that diphtheria in the acute stages can involve the conduction apparatus and produce heart block. Our study lends further support to the view that diphtheria in childhood may be an etiological factor in the development of heart block in later years.

Changes in Cardiac Action During Attacks of Angina Pectoris (Clinical and Experimental Observations). By FRANCIS C. WOOD, (by invitation) and CHARLES C. WOLFERTH, Philadelphia, Pa.

Twenty-seven cases of angina pectoris were studied, by means of the electrocardiograph, before, during and after attacks, in an attempt to determine whether there was evidence of some specific change in cardiac action during the attack. Six attacks were spontaneous, 21 were induced by prescribed exertion. As a basis for comparison, the effect of exercise on the electrocardiogram was studied in 160 normals, 25 patients with non-anginal cardiovascular disease, 9 cases of angina in which exercise was not productive of an attack, and 2 patients with non-cardiac pain.

Fourteen of the 27 cases of angina pectoris showed bizarre electrocardiographic changes during the attack, unlike anything seen in the non-anginal cases. Thirteen showed no characteristic alterations during the pain.

The types of electrocardiographic changes, their complete reversibility, and the rapidity of their onset and disappearance were compared with electrocardiograms of dogs with temporary coronary occlusion. A close parallelism was observed.

Anoxemia of a section of a dog's myocardium due to coronary occlusion does

not always alter the electrocardiogram. Therefore, the absence of significant change in the human electrocardiogram during an attack of angina pectoris cannot be used as evidence that no anoxemia of the myocardium occurred.

The mortality has been overwhelmingly greater in the group with electrocardiographic changes during the attack than in the group without them. This might be considered as added evidence that the bizarre electrocardiograms observed during some anginal attacks are dependent upon an important and significant change in the condition of the heart.

It is concluded, therefore, that the electrocardiograph gives evidence of some unusual change in cardiac action during a certain percentage of anginal attacks, and that the evidence is at least suggestive that this change is due to anoxemia of a section of the myocardium.

The Application of Schütz' Law in Clinical Pepsin Determinations. By J. HAROLD AUSTIN and (by invitation) GEORGE D. GAMMON.

The method of Bloomfield and Pollard for pepsin determination has been found useful. The data obtained with either pure pepsin solution or gastric juice conforms approximately to Schütz' law when from one-third to two-thirds of the total edestin has been digested. This fact offers a means of calculating the data into terms of pepsin concentration which is in our hands both more reliable and simpler than the method of calculation suggested by Bloomfield and Pollard and appears to us to have a sounder theoretical basis.

The Measurement of Sympathetic Vaso-constrictor Activity in the Lower Extremity.

By J. J. MORTON and W. J. MERLE SCOTT, Rochester, N. Y.

Vascular diseases in the lower extremity are dependent on two mechanisms; (1) organic occlusion, and (2) functional spasm. For the prognosis and treatment of such conditions it is important to determine the relative participation of these two elements. No entirely satisfactory method for this differentiation is available at present. The skin temperature is used as an index of the circulation to the part.

After spinal anesthesia an increase in the temperature of the foot up to 12°C. is at times found. The elevation of surface temperature is associated with a flushing of the skin, and is coincidental in time with the appearance of muscular paralysis. It is limited to that part of the body, the motor nerves to which are blocked by novocaine. In purely occlusive vascular disease and in one group of patients without obvious vascular abnormality (particularly cachectic individuals) there is no increase in the surface temperature. We propose the increase of surface temperature in the lower extremity following spinal anesthesia as a measure of the sympathetic vaso-constrictor activity at that point.

A Study of Subcutaneous Nodules in Chronic Infections (Rheumatoid) Arthritis.

By M. H. DAWSON, and R. H. BOOTS, (by invitation) and W. W. PALMER, New York, N. Y.

The occurrence of subcutaneous nodules in patients suffering from rheumatic

fever has long been recognized and has been the subject of intensive investigation for many years. Carey Coombs has stated that "of the principal phenomena of orthodox rheumatic infection—namely, carditis, polyarthritis, the subcutaneous node, and chorea—the subcutaneous node is the most rheumatic of all." Swift and other investigators have, from time to time, expressed similar views.

Considerably less attention, however, has been paid to subcutaneous nodules occurring in patients suffering from chronic infectious arthritis. During the past year such nodules have been observed in thirty-three patients in the Arthritic Clinic of the Presbyterian Hospital. Nodules have been removed from ten patients and subjected to pathological and bacteriological investigation. All the specimens examined have shown a distinctive and characteristic histological appearance. The various stages in the development of the lesion have been studied and a remarkable resemblance to the nodules of rheumatic fever has been demonstrated. In fact the evidence suggests that the nodules in the two diseases represent different stages in essentially the same pathological process.

The bacteriological investigations have been entirely without result.

The relationship between rheumatic fever and chronic infectious arthritis has also been studied clinically in a group of 300 arthritic patients. The conclusions drawn from these studies also suggest that the two diseases are very closely related and may represent different stages of the same inflammatory process.

Uremic Stomatitis. By PHILIP S. HENCH and (by invitation) BERT E. HEMPSTEAD, Rochester, Minn.

Stomatitis associated with uremia is not described in the English literature, and has been described only three times heretofore, in two French reports and one South American.

Four cases are reported from the Mayo Clinic, with clinical and pathological details. The possible causes of uremic stomatitis and the relation of the oral lesions to uremic ulceration of the intestines and skin are discussed. The various theories regarding uremic stomatitis in general are also discussed.

Uremic stomatitis is a rare condition, but one which it is important for the clinician to recognize as it is of definite diagnostic and prognostic value.

The Clinical Picture of Follicular Lymphoblastoma. By GEORGE BAEHR, New York, N. Y.

A distinctive variety of lymphosarcoma, characterized by the tendency to reproduce lymph follicles of huge size in lymph nodes and spleen is described. The giant follicles consist of greatly enlarged germinal centers composed of lymphoblasts surrounded by a narrow zone of darker staining mature lymphocytes. They crowd one another so as to compress the intervening sinuses and pulp. The huge spleen, weighing in one case 1800 grams, is thickly studded with follicles the size of barley grains.

The clinical picture is distinguished by the prolonged clinical course and relative benignity, the diffuse involvement of spleen and lymphatic apparatus throughout

the body, the tendency to thoracic and abdominal effusions, the frequency of unilateral exophthalmos due to involvement of the lachrymal gland or periorbital fat, the absence of cachexia or profound anaemia and the normal blood picture.

The ultimate differential diagnosis is dependent upon microscopic examination of a lymph node removed by biopsy. Histologically, it is sometimes difficult to distinguish from simple hyperplasia. In fact the first three cases were described in 1925, in conjunction with Drs. Brill and Rosenthal, as "Giant Lymph Follicle Hyperplasia of Lymph Nodes and Spleen."

Aside from the distinctive clinical and pathological features, its clinical recognition is important because of its remarkable roentgen sensitivity, the splenomegaly and enlarged lymph nodes promptly melting away under moderate x-ray therapy. There is always tendency, however, for the neoplasm to recur in distant parts of the body, though sometimes only after several years.

The chronicity of the disease under treatment is illustrated by three cases followed throughout most of their clinical course. One died nine years after the onset, another after five years, the third is still alive and apparently well after ten years. Death eventually occurs because the lymphatic enlargements become more resistant to treatment or because the neoplasm is located in sites not easily reached by the x-ray.

Diabetes Mellitus and Pernicious Anemia. By HOWARD ROOT, Brookline, Mass.

The occurrence of 12 cases of combined diabetes and pernicious anemia at the Deaconess Hospital during the last two years led to a search of the literature and finding a total of 32 such cases. A striking feature of the series is that in nearly all cases where accurate histories were available the diabetes preceded the anemia in onset. The clinical features of the two diseases were characteristic, but in certain instances the combination of diseases produced interesting findings. In one case the effect of fasting treatment and the development of diabetic acidosis was to cause a very rapid progress of the anemia and a decrease in the red cells from 3.0 to 1.2 millions in 10 days. An apparent resistance to insulin during the height of the anemia was observed in another case. The rapid development of anemia in a diabetic with a septic leg illustrated the effect of sepsis and diabetes upon a mild and early case of pernicious anemia. A case with advanced symptoms of combined system disease made surprising functional improvement with a combination of dietary, insulin, iron and orthopedic treatment.

In seeking a possible relationship, we determined the hemoglobin of the blood routinely and in patients with diabetes of more than 5 years duration undergoing hospital treatment found a slight reduction in nearly all. The blood smears showed slight variations in the size and shape of red cells and abnormality of the platelets. Cases in this group, however, who had absence of free HCl in the gastric secretion had reduced red blood counts with much more marked change in the smears. Certain cases of the latter group, under treatment including the use of iron and liver for 2 or more years, have not had relapses or developed symptoms of anemia. The increasing duration of life both of diabetics and of cases of pernicious

anemia may result in greater frequency of this association merely because the mathematical chances of the association are thus increased, but the earlier onset of the diabetes, and the effect of diabetic treatment upon the anemia suggest that diabetes itself, especially when achlorhydria is present may prepare the way for pernicious anemia.

Some Observations upon the Physiologic and Therapeutic Action of Glycocyamine.

By RALPH H. MAJOR, Kansas City, Kans.

Glycocyamine is guanidine acetic acid and differs from creatine only in the absence of the methyl group. Creatine has no effect upon the blood pressure. Glycocyamine has a marked depressor effect.

Observations on capillaries and arterioles show a dilatation after injection of glycocyamine. Observations on the coronary circulation show a very marked increase in coronary flow with a small dose. If the dose is increased to the point of producing a very sudden and intense fall in blood pressure, the coronary circulation is diminished.—The relationship of these observations to the course of treatment in arterial hypertension is commented upon.

Appearance of Protective Antibodies following Intravenous Injection of Pneumococcus Vaccine in Lobar Pneumonia. By ALVAN L. BARACH, New York, N. Y.

The intravenous injection of an antigenic filtrate of the pneumococcus resulted in the appearance of protective substance in the serum of nine patients, seven of whom had lobar pneumonia, on the sixth day and occasionally on the fifth day after injection. After the injection of the intact organism, protective substance appeared on the fourth, and at times on the third day after injection. The immunity increases progressively for three days following injection. This applies to pneumococcus types I, II, and III, and has been produced during the febrile period of the disease.

Clinical Significance of a Large Q Wave in Lead 3. By HAROLD E. B. PARDEE, New York, N. Y.

A large Q wave in the third lead has been observed in records from patients with the anginal syndrome. The frequency of occurrence in such cases is about 27 per cent. Similiar records are obtained from certain patients with rheumatic heart disease, especially those with pericarditis, from a few patients with myocardial fibrosis and congestive heart failure and a few with hypertension without the congestive anginal syndrome. A record with similar large Q waves in lead 3 was found only twice in two hundred and seventy-seven records from apparently normal hearts. In addition to the Q wave of lead 3, these records show a clockwise rotation of the vectors of the QRS group. They usually show a left axis deviation of QRS and frequently there is an inversion of the T wave in lead 3 or in leads 2 and 3. A discussion of the origin of these features leads to the conclusion that the large Q-3 is due to disease of the muscle of the left ventricle. It is observed, however, that a high position of the diaphragm may be a contributory factor in the

production of this peculiarity, and that in the normal heart there may be a peculiar distribution of the branches of the auriculoventricular bundle.

The Relationship between the Specific Gravity of the Urine and the Functional State of the Kidneys. By F. H. LASHMET, (by invitation) and L. H. NEWBURGH, Ann Arbor, Mich.

After the subject has been in bed for three days on a standard diet, all food and drink is omitted at 6:00 p.m. of the third day. Thereafter a standard waste in the presence of a limited supply of water is presented to the kidneys for excretion. Normal subjects, eighteen hours later void urine whose specific gravity is 1.025 or more. Nephritic subjects, under the same conditions, have a specific gravity as low as 1.005. Correction for temperature is avoided by the use of a pycnometer.

Albumin raises the specific gravity in proportion to its concentration. For example, the specific gravity of the concentrated urine of a nephritic subject was 1.021 before the correction for albumin but only 1.010 after correction. The urine contained 2.5 per cent protein.

If the specific gravity, the blood N.P.N. and phenosulphonephthalein test are compared, it is found that in a group of eighty-six cases with evidence of kidney damage, the blood N.P.N. was normal in 79 per cent; the phthalein test was normal in 30 per cent; but only 10 per cent concentrated the urine specific gravity to 1.025 or above.

We conclude that when the specific gravity of the urine is obtained under the above conditions, it is a much more accurate test of the functional state of the kidneys than the phthalein test or blood N.P.N. and that a roughly quantitative idea of the amount of incapacity may be obtained in this simple way.

Studies on the Colloid Chemistry and Ultramicroscopic Phenomena in Antisepsis and Chemotherapy. By ARTHUR D. HIRSCHFELDER and (by invitation) HAROLD N. WRIGHT, Minneapolis, Minn.

In order to discover, if possible, some of the general principles underlying the phenomena of antiseptics and chemotherapy, we have studied the colloid chemical aspects. We have found that mercurochrome and acriflavine are semi-colloids. The triphenyl-methane dyes are crystalloid, and their fixation by egg albumin, as shown by dialysis experiments, follows the logarithmic curve, which indicates adsorption rather than chemical combination. The fraction absorbed on egg albumin retains some antiseptic action.

The acquired drug fastness toward trypanosomes produced by Browning and Schnitzer with triphenyl-methane dyes against acriflavine, and vice versa, can be duplicated on yeasts *in vitro*. We believe that this is a surface reaction.

Under the ultramicroscope, particles of lyophilic colloids, like egg albumin, look like soap bubbles; lyophobic colloids look like bright stars and comets. Addition of various antiseptics to egg albumin converts the picture into star and comet appearance like that of a lyophobic colloid. Plasma proteins are better protected but these drugs produce similar, though less marked changes, *in vitro* and *in vivo*. Mercurochrome intravenously is fixed by plasma colloids and alters them visibly.

These studies throw some light on toxic reactions of drugs and other pathological phenomena. We propose to investigate these questions.

Observations on the Intelligence Quotient from the Physician's Viewpoint. By H. GRAY, San Francisco, Calif.

Behavior problems and physical diseases in children are less often associated in the physician's mind than they might be. Though no neurological residuals are apparent, there occur at times changes in the brain which the medical man might detect by turning to the intelligence tests in use by psychologists and psychiatrists. In the Institute for Juvenile Research in Chicago, to which are referred children with behavior problems, a search was made for medical diagnoses.

Encephalitis was found in 89 patients with an average IQ of 90.6, which (compared with the average of 90.3 for 2815 cases of all diagnoses) was not significant. If however we consider of this group only those who had mental deterioration, by taking as demarcating line a twelve point drop in IQ, we find 24 cases, and on examining their records for possible medical cause we find three with a history of encephalitis, namely 12 per cent. It is accordingly suggested that a physician who has the care of a child with encephalitis inquire whether an IQ has been determined prior to the illness and if so that he secure a repetition after apparent recovery.

Syphilis was found in 156 children, with an average IQ of 87.8 which (again compared with the average for all diagnoses as stated above) is significantly low.

Delinquency problems are said not to occur in children with IQ's above 130. In this material however 35 such cases are present. Whence it may be urged that children of intelligence must not be considered immune to behavior difficulties.

The physician is sometimes called upon to advise with the school principal as to whether a given child should be pushed up a class or held back. Intelligence quotients are more rapidly obtained, and more standard as between schools, than teachers' marks. Further it is not sufficiently known that children in private schools show distinctly different IQ's from children in public schools, on whom the current standards have been based. In a private school the IQ on 179 children aged 5 to 19 years averaged 114, namely 14 per cent higher than the usual norm of 100.

The Mechanism of the Epigastric Distress Associated with Extragastric Lesion. By FRED M. SMITH, and (by invitation) WM. D. PAUL, and W. M. FOWLER, Iowa City, Iowa.

The epigastric distress under consideration is usually localized to the right, and slightly above the umbilicus, and frequently appears from 1 to 3 hours after meals. It is characterized as a feeling of fullness, heaviness, burning, or a gnawing sensation. The site of the distress on fluoroscopic examination, corresponded with the pyloric portion of the stomach and shifts with the change in the position of this region. The association of this distress, with discomfort elsewhere in the abdomen, suggests a reflex stimulation of the stomach. In a former investigation, a reflex

stimulation of the stomach from gallbladder, appendix and colon was demonstrated in the dog. A consideration of this possibility in man was then investigated. Patients with a spastic colon and a recurring epigastric distress were first selected for study. A balloon was introduced in the pyloric portion of the stomach and connected with a kymograph. After a control record of the gastric activity had been obtained, the colon was distended with air through a rectal tube. There was at once a striking increase in tone and peristaltic action of the stomach, and the appearance of the typical epigastric distress. The pain corresponded with change in tone or the passage of a peristaltic wave over the pyloric portion of the stomach. This induced alteration in the stomach, and the distress promptly subsided after deflation of the colon, or was abolished by atropin. In a patient with chronic appendicitis, similar changes in the stomach, and the typical epigastric distress was induced by massage over the ileocaecal region. This patient was fluoroscoped during the palpation of the appendix region and a striking increase in tone and peristaltic action of the stomach and the appearance of a marked prepyloric spasm was observed and recorded on films. The epigastric distress was experienced during the massage over the appendix, and corresponded with the site of the prepyloric spasm. Similar roentgenological observations were made on patients with an irritable colon. The type of epigastric distress studied is gastric in origin. It is produced by an increase in tone and peristaltic action of the stomach, which is induced by a reflex stimulation from the colon and appendix.

Hemodynamic Studies in a Case of Concretio Cordis. By C. SIDNEY BURWELL and (by invitation) W. D. STRAYHORN, JR., Nashville, Tenn.

The patient exhibited signs of obstruction to the flow of blood into the right heart. He had ascites, pleural transudates, peripheral edema, an enlarged liver, distended veins, and a paradoxical pulse. The heart was small, fixed in position and showed no pulsation when observed with the fluoroscope nor any change in electrical axis with shift of position. The sounds were feeble, the pulse small. The lungs exhibited no râles, and the arterial blood was 98 per cent saturated with oxygen. The absence of pulmonary congestion made feasible the measurement of the cardiac output by the acetylene method of Grollman. The venous pressure was measured by the method of Moritz and Tabora.

Under standard "basal" conditions the following observations were made: low pulse pressure (94/82 mm. Hg), rapid pulse (104-110), low cardiac output per minute (1.98-2.72 liters), very low output per beat (18-26 cc.), high oxygen utilization (9.0-11.8 volumes per cent, 53-70 per cent utilization), and venous pressure of 240 mm. H₂O. The venous pressure rose rapidly with the slightest muscular exertion.

When the oxygen consumption per minute was doubled by exercise the following observations were made:

The pulse pressure and output per beat were unchanged, the pulse rate rose to 144, the cardiac output increased only in proportion to the rate, the oxygen utilization rose to 13.0 volumes per cent (77 per cent utilization), the venous distension was obviously greater, and the patient was clearly cyanotic.

After digitalis (1.6 grams) the pulse rate fell to 90, the cardiac output per minute fell in proportion, the output per beat remained unchanged, and the patient felt less comfortable.

Operation was followed in 12 hours by sudden death. Autopsy revealed a small heart with atrophy of the ventricular myocardium, the whole surrounded by a dense capsule of fibrous tissue which was not adherent to the chest wall but which must have effectually prevented adequate diastolic relaxation.

The symptoms were due to back pressure rather than to diminished volume flow because stasis occurred only in the systemic circulation. The peripheral signs were those of heart failure, the signs in the heart itself were in general the precise opposite of those observed in characteristic congestive heart failure. These observations thus have important bearing on the problem of the mechanism of heart failure.

An Undescribed Cardiac Mechanism: "Intraventricular Block" with Short P-R Interval in Healthy Young People Prone to Paroxysmal Tachycardia. By LOUIS WOLFF, JOHN PARKINSON (by invitation) and PAUL D. WHITE, Boston, Mass.

Eleven cases are here reported of an unusual cardiac mechanism, heretofore undescribed as such, consisting of functional bundle branch block and abnormally short P-R interval, occurring mostly in otherwise healthy young people with paroxysms of tachycardia or of auricular fibrillation.

Spontaneously, or following release of vagal tone by exercise or atropinization, the ventricular complexes revert to the normal physiological form, and the P-R interval lengthens (normal).

Vagal influences seem to be largely responsible for the mechanism described. A paradoxical effect of vagal stimulation on the P-R interval has been observed.

From a study of the cases presented it is concluded that:

- a. Aberrant ventricular complexes of the type generally recognized as indicating bundle branch block may occur in healthy people with normal hearts.
- b. Vagal stimulation is capable of altering markedly the form of the ventricular complex, and may be responsible for the occurrence of complete bundle branch block curves in apparently normal hearts at normal rates of beating.
- c. Vagal stimulation may shorten markedly the P-R interval without the production of A-V nodal rhythm, and without dislocating the auricular pacemaker.
- d. In the group of cases reported paroxysmal tachycardia and auricular fibrillation are obviously associated with the nervous control of the heart.

Reactions After Administration of Crystalline Insulin. By W. R. CAMPBELL and (by invitation) W. J. GARDINER, and D. A. SCOTT, Toronto, Can.

Crystalline insulin was administered in sufficient amounts to patients to induce a lowering of blood sugar. The symptomatology was studied in relation to the level of the blood sugar. As was previously observed, the level at which symptoms appears is about 0.070 per cent. Patients not reaching this level were without

symptoms. In some instances at this level symptoms were slight and would probably have been overlooked by the patient. No patients reaching hypoglycaemic levels failed to report symptoms and these were more marked with the lower blood sugar levels. In comparison with the crude insulin of 1922 and even the present commercial insulin, reactions are less marked with the crystalline product. No constant effect was noted on the pulse rate or blood pressure; in most cases the values remained unchanged.

Certain patients present initially or develop after a short period of treatment local sensitization phenomena at the site of the injection of insulin. When of minor degree, the patient desensitizes himself. When more severe, it is necessary to change the source of the insulin. One patient, however, shows marked sensitivity to beef, hog, sheep, fish and human insulin obtained from different sources. He is also sensitive to crystalline insulin though the reaction is less intense. The sensitivity can be locally communicated to other individuals by passive transfer. He has now developed local desensitization on a part of one thigh for crystalline insulin only. Other patients are sensitive to insulin from two or more species and also to crystalline insulin several times recrystallized but the sensitivity in all cases is less marked to crystalline insulin than to other commercial insulins. Inasmuch as the patients are not sensitized to beef muscle, hog muscle, etc., there is probably a sensitization to organ protein as well as to the insulin protein.

Local Immunity in Areas of Skin Hypersensitiveness. By FRANKLIN M. HANGER, JR., New York, N. Y.

Rabbits recovering from a skin infection of *B. leprosepticum* are highly immune to re-infection by these organisms. The serum of such animals will protect a local area of skin of a non-immune animal when injected either previously or simultaneously with an emulsion of virulent *B. leprosepticum*.

We have been interested in studying the effect of local hypersensitive reactions to heterologous proteins on the efficacy of this immune mechanism.

Immune and non-immune rabbits were sensitized to egg albumen by repeated subcutaneous injections. After several weeks, skin sensitivity to egg developed as manifested by the usual raised, red, oedematous areas at the site of intradermal injection of 0.1 cc. of egg albumen twenty-four hours previously.

When a thin suspension of *B. leprosepticum* is injected into these areas the infection spreads rapidly throughout the entire wheal in contrast to the much smaller lesion in control normal skin. Even in immune animals with highly protective serum a spread of infection is observed when the local reaction to egg is intense.

When wheals are produced by injecting egg and immune serum simultaneously, infection spreads unchecked. However in non sensitized animals, areas of skin receiving an identical egg-immune serum mixture show complete protection. It is suggested that the serological factors of immunity may be greatly altered by local cellular conditioning.

Fluorescence of the Skin as a Protective Mechanism in Pellagra. By ROY H. TURNER, New Orleans, La.

Sunshine is generally admitted to be the most important precipitating cause of the dermatitis of pellagra. The ultraviolet portion of the spectrum has recently been shown to be the most injurious. The observations to be reported were undertaken in an effort to explain instances of apparent immunity in pellagrins to injury by sunshine. The face, though the most exposed to light, is rarely injured and then only mildly. Skins with thick, horny layers seem protected. Castle has shown that keratin and sebum are fluorescent. This quality makes them protective against ultraviolet light, since these rays are changed to visible rays in passing through fluorescent substances.

A series of normal persons and pellagrins have been examined in a dark room by ultraviolet light. Normally the most fluorescent areas are the palms, soles and face, which are areas remarkably free from the dermatitis of pellagra. Early skin lesions of pellagra were non-fluorescent, while the later lesions with roughened skins were highly fluorescent. Lesions at this stage have been shown very resistant to light. Experiments to show that sebum and keratin are the fluorescent substances will be reported.

The Effect of Ergotamine on the Response to Adrenalin in Normal Human Subjects under Basal Conditions. By JOHN B. YOUNG and (by invitation) W. H. TRIMBLE, Nashville, Tenn.

Our previous studies have shown that under basal conditions ergotamine, in doses of 0.5 mgm. subcutaneously, has no significant effect on the metabolic rate or blood sugar level of normal human subjects but causes a small drop in pulse rate and a slight rise in blood pressure. In the present study we have found that under similar conditions ergotamine tends to diminish or delay the increase in metabolic rate and blood sugar which results from the injection 0.5 to 1 mgm. of adrenalin. Ergotamine had little or no effect upon the increase in heart rate caused by adrenalin but augmented slightly the blood pressure raising effect of the latter.

The Response of Blood Platelets to the Administration of Viosterol. By L. D. THOMPSON (by invitation) and D. P. BARR, St. Louis, Mo.

An increase in the number of platelets following exposure of experimental animals to sunlight or ultraviolet light has been noted by a number of investigators (Cramer and Drew, Laurens, Sooy and Sanford). Other observers have noted changes in coagulation time following the administration of cod liver oil (Brougher) and viosterol (Selye) but have made no note of increase of platelets. Phillips and Robertson of Washington University reported an increase in platelet count in the rat following administration of viosterol. Our study was undertaken to determine the effect of viosterol upon the platelet count of man under normal conditions and the presence of certain diseases.

A single oral dose of viosterol causes a slight rise in platelet count in twelve to twenty-four hours which is followed by a fall to approximately normal and a

secondary rise reaching a maximum on the fourth to fifth day. The maximum count varies from 600,000 to 900,000. The decline is sharp and the number of platelets reaches normal limits by the sixth to eighth day. The response to single doses of 7, 15 and 22 minims is essentially the same. A similar response can be obtained a second time in a given individual after an interval of fourteen days. The response to daily doses of 10 minims differs from that following the single dose. Both the rise and decline in the number of platelets are more gradual but the maximum is reached at about the same time, namely five to seven days. On the ninth to eleventh day the count is within normal limits and continues so for at least three weeks except for irregular, transient increases of 100,000 to 200,000. The bleeding time and coagulation time are decreased coincident with the increase in platelet count.

In splenectomized individuals, the spleen having been removed 18 months to 6 years previously, the response to viosterol is exaggerated. After the administration of a single dose, the rise in platelets is sharp and continuous and reaches a maximum of 1,400,000 to 1,600,000. The decline is also more rapid than normal.

In hemophilia, an approximately normal response to daily doses has been seen. Even in the presence of 800,000 platelets, however, there has been no change in symptoms, bleeding has continued and the red blood cell count has readily fallen.

In thrombocytopenic purpura, the platelet count, the coagulation time and clot retraction may be held within normal limits by viosterol but showers of purpuric spots and hemorrhages from mucous membranes have continued.

Studies have been made and are being continued on a variety of blood dyscrasias. The presence of jaundice does not prevent the normal platelet response to viosterol. In aplastic anemia, the platelets may rise above normal at a time when the number of red and white blood cells is rapidly falling.

The Effect of Sodium Salicylate on Intradermal Reactions of Rabbits. By O. E. HAGEBUSH, (by invitation) and R. A. KINSELLA, St. Louis, Mo.

Sodium salicylate is commonly used in the treatment of infections, especially those presumed to be due to invasion by streptococcus. The old idea that acute rheumatic fever is due to infection by streptococcus, and the recently developed conception that the disease is involved in a process of allergy to streptococcus, stimulate a study of sodium salicylate in relation to allergy to streptococcus.

In this study rabbits were inoculated with cultures of a strain of *Streptococcus hemolyticus* of low virulence. The injections were made into one of the knee joints, 0.1 cc. of broth culture being used.

For intradermal tests, 0.1 cc. of filtrate from a 5-day culture in Harley's medium was used. Areas near the spine were used for inoculation.

All animals were tested for native reactivity to the filtrate before being employed in the experiments. Fresh animals giving positive dermal reactions were discarded.

In the use of sodium salicylate, 0.2 gram per kilo in 5 per cent aqueous solution was given intravenously. The injections were made more slowly and given daily.

Preliminary studies showed that following the intra-articular injection of streptococci, purulent arthritis invariably resulted and persisted until the death of the animal. Blood cultures were rarely positive during the life of the animal unless an intercurrent disease such as "snuffles" intervened. When this occurred, hemolytic streptococci were found in the blood at autopsy. Ten days after arthritis was established, positive intradermal reactions were always present.

The conditions of the experiment were severe due to the nature of the infection and the death rate was high in all series. This did not seem to be considerably influenced by the use of sodium salicylate.

In the first series of animals 15 controls gave strongly positive intradermal reactions 10 days after the production of arthritis, and 8 animals, given sodium salicylate 24 hours before the production of arthritis and at 24 hour intervals thereafter, showed slight or no intradermal response.

At this point the results of studies made by Griffin of the reciprocally antagonistic action of benzoic acid and glycine on the growth of white rats, were considered. According to this author the deleterious effect of benzoic acid on the growth of young white rats was removed by adding glycine to the diet. The amount of glycine used was 0.47 gram per kilo, in 4 per cent aqueous solution.

Groups of rabbits were therefore selected, of which one was composed of controls, one of animals receiving sodium salicylate intravenously, one of animals receiving glycine intravenously and one of animals receiving sodium salicylate and glycine freshly mixed in proper solutions. In this experiment, 16 control animals gave strongly positive reactions; 28 animals receiving sodium salicylate, gave slight or no reactions; 4 animals receiving glycine alone gave strongly positive reactions; and 16 animals receiving mixtures of glycine and sodium salicylate, gave strongly positive reactions.

This effective suppression of so-called allergic reactions by sodium salicylate depends on factors as yet unknown. There is no reason to ascribe this effect to a general depressive action inasmuch as rabbits did not lose weight or appear less healthy while receiving sodium salicylate over a period of 10 days than did controls.

Since the dermal reaction is not heightened by the use of glycine alone, the dermal responses obtained in animals treated with sodium salicylate and glycine combined, must be present because glycine neutralizes sodium salicylate *in vivo*.

The nature of the dermal reaction itself remains obscure. Whatever the mechanism by which sodium salicylate suppresses the dermal reaction, this mechanism does not effect the nature of the vascular pathology found in these animals. The late proliferative vascular lesions were the same in the various series.

When sodium salicylate was discontinued the dermal reactions tended to return, but after 20 days in 7 animals, only a slightly positive reaction was present, while after 50 days only a moderate reaction was present in 4 surviving animals. When sodium salicylate was administered to 4 animals which had well developed dermal reactions the effect was a gradual suppression of the dermal reaction.

The experiments thus indicate that salicylates would be more effective in preventing the development of an allergic state following a primary infection than in removing allergy after the primary infection has fully developed.

If acute rheumatic fever is a disease in which the process of allergy is involved then derivatives of salicylic acid might conceivably be beneficial by interfering with this process of allergy. That this interference is of too little importance to modify the vascular pathology is apparent. By implication the overlying process of allergy is unimportant and the underlying focus of infection, at present reputed to be of streptococcal origin, is very important. In turn, the results of cultures of blood and joints become of great importance—a state of the question now many years old.

From these considerations it seems possible to draw the following conclusions:

1. Sodium salicylate suppresses the allergic dermal reactions of rabbits to filtrates of hemolytic streptococcus. This effect is most definite when sodium salicylate is given before the focus of infection has developed.
2. There is no relation between the presence or absence of this dermal reactivity and the character of the vascular pathology.

THE ASSOCIATION OF RENAL AND GASTRIC DISORDERS WITH CONSTANCY OF THE URINARY REACTION

By ROGER S. HUBBARD

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New York)*

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It has been said that determinations of the alkaline tide in urine are of value in the study of two entirely distinct clinical conditions. One of these is associated with diseases of the stomach. Bence-Jones (1, 2) who seems to have been the first to describe diurnal variations in the acidity of urine, believed that they were associated with the secretion of acid into the stomach. Many physiologists have held similar views, (3, 4) and a number of different workers have suggested that an absence of changes in the reaction of urine means that the stomach does not secrete hydrochloric acid, (5, 6, 7).

Leathes, (8, 9) McCorvie, (10) and others have taken an entirely different view of the problem. They believe that disease of the kidneys is usually, if not always, responsible for an absence of the alkaline tide. According to them relative over-ventilation through the lungs is the cause of the rhythmical changes in the reaction of the urine. When the kidneys are diseased the urine fails to reflect the changes in the blood induced by hyperpnoea, and its reaction is relatively constant. There seems to be no extensive series of cases in the literature in which the relationships of urinary reaction to both gastric and kidney abnormalities are considered in the same patients. Such a study is presented in this article.

Alkaline tide determinations have been made in this institution upon 296 subjects by the following technique. At seven o'clock the patient was aroused, and the night urine voided and, in most instances, discarded. Specimens were collected at hourly intervals thereafter until one o'clock. The reaction of these was determined by a colorimetric method. A meal, usually consisting of a glass of milk, a glass of water, two slices of toast with butter, and an egg, was fed between

eight and nine o'clock. Most of the subjects were suffering from gastric disorders, and in many instances the presence of achylia gastrica was suspected.

The present discussion is based upon 173 of these patients who failed to show an alkaline tide as defined by Munford and Hubbard (5). These authors consider that a tide is present when the reaction of any specimen is more alkaline than that of one collected earlier in the experimental period by 1.0 pH, or when the reactions of any two specimens are more alkaline than a previous one by 0.5 pH. This definition of a tide certainly cannot be considered wholly satisfactory, but it serves as a convenient method for separating those tests in which there was at most a slight tide from those in which there was a more definite one.

The clinical histories and laboratory findings of these 173 patients were reviewed to determine the incidence of gastric and renal disorder. Eleven histories were not available. Gastric analyses were done upon 118 patients. In 78 hydrochloric acid was absent from the gastric juice; in 15 there was a marked hypochlorhydria, and only 25 showed an approximately normal concentration of acid after the test meal.

Among 162 patients whose urine showed little or no tendency toward the development of alkalinity during the morning there were many cases of achlorhydria and hypochlorhydria. What was the incidence of kidney disease in the series? In only 24 instances did the diagnoses suggest that circulatory or renal failure might have been present. These diagnoses included two cases of rheumatic and eleven of arteriosclerotic heart disease. Of the latter group two showed signs of congestive failure and three had auricular fibrillation. There were three cases with diagnoses of chronic nephritis, one of which was associated with hypertension and arteriosclerosis. Hypertension was diagnosed four times and arteriosclerosis three times. One diagnosis read pernicious anemia with heart block. It is evident that marked cardiovascular-renal disease occurred rather infrequently.

This evidence is supported by the available determinations of urea in the blood and of the rate at which phenolsulphonephthalein was excreted. The former study was carried out 27 times. The average value was 16.5 mgm. of urea nitrogen per 100 cc. of blood. In only four patients were values greater than 20 mgm. found. The phenol-

sulphonephthalein test was done on 43 patients. The mean value of these was 50 per cent. No patient excreted less than 28 per cent of the dye in two hours, and only 7 of them less than 40 per cent during that period. More than half of the subjects excreted over 50 per cent—a figure which should almost certainly be considered normal when the dye is given intramuscularly and the urine is not collected through a catheter.

It seemed worth while to take into consideration the blood pressure readings of the group. The average values were systolic 135 and diastolic 76 mm. There were only 7 instances in which the systolic pressure was over 180 and the same number with diastolic readings over 100 mm. The figures certainly are not high.

Recently (11) it has been shown that there are two separate periods of increased urinary alkalinity during the morning. One of these occurs before any meal is fed and is probably due to respiratory adjustment to the activities of the day. The other develops later in the morning, and is caused, according to the author's view, by the secretion of hydrochloric acid into the stomach. The method by which the cases in this series were selected has confined the study to those showing an absence of the second tide. The results have also been analyzed to determine the relationship between nephritis and a urinary alkalinity developing before any meal is fed because the work of Leathes (8, 9) and McCorvie (10) suggests that such a study may be profitable. For this purpose those cases in the series already discussed in which the reaction of the night urine was determined have been considered separately. Such figures were available for 130 patients. In 15 of them the value of the pH representing the night urine was 7.0 or higher. These tests have been discarded because development of further alkalinity under such conditions seems improbable. Forty-five showed a respiratory tide as defined by the author; that is the night urine was more acid by at least 0.5 pH than that collected immediately after awakening. The remaining 70 patients failed to show either the gastric or respiratory tide.

The incidence of achlorhydria among these 70 subjects was approximately the same as in the whole series. Kidney disease, however, seemed to be present somewhat more frequently in the smaller group. Although less than half of the cases showed an absence of the early

morning period of alkalinity this group provided seventeen of the twenty-four diagnoses which suggest the presence of cardiovascular-renal disease. These seventeen included all three of the cases of chronic nephritis, six of those with arteriosclerotic heart disease, four with arterioclerosis, and three with hypertension. However, since there were 53 cases out of the 70 for whom no such diagnoses were made, the difference between the groups with and without early alkaline tide is not striking. The routine urine findings agreed well with the clinical diagnoses. There was a somewhat higher incidence of marked abnormalities in the series without early alkaline tide, for five of the six patients who excreted more than a trace of albumin were included. Two of the four patients with red blood cells and 15 of the 27 with casts also showed no early period of alkalinity. On the other hand 28 of these seventy patients showed nothing abnormal in the urine at any time, and 10 others excreted only an occasional faint trace of albumin. Blood urea determinations, studies of the rate at which phenolsulphonephthalein was excreted, and blood pressure readings were almost exactly the same in the small series of 70 as they were in the large one of which it formed a part.

In a series of cases showing an absence of the characteristic alkaline tide after a meal achlorhydria occurred rather frequently, but there was little evidence of the presence of cardiovascular-renal disease. An absence of the early morning respiratory tide was somewhat more frequently associated with the conditions last named, but in a large proportion of the cases which showed no change of any sort in the urinary reaction there was no sign of renal or circulatory involvement. Since, however, the cases studied were picked from those in whom the presence of an abnormal gastric condition was suspected, and not from those showing lowered kidney function, care must be taken in drawing conclusions from the results. The conclusions may properly be stated as follows: 1st, relative constancy in reaction of the morning urine is often associated with an absence of hydrochloric acid from the gastric juice; 2nd, constancy in the reaction of the morning urine frequently occurs when no impairment of renal function and no signs of kidney disease can be demonstrated; 3rd, although there is some evidence that an absence of the early morning period of alkalinity may be associated with diseases of the kidneys the relationship is not an essential one.

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THE PNEUMOCOCCIDAL POWER OF WHOLE BLOOD

I. DESCRIPTION OF METHOD. RESULTS IN INDIVIDUALS WITH NO HISTORY OF LOBAR PNEUMONIA¹

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The immunological properties of whole blood in relation to the pneumococcus have been studied by various methods. Wright, A. E. (1) and his followers used the slide cell technique devised by him and based upon the use of defibrinated blood. Heist and his co-workers (2, 3) have also used defibrinated blood. Bull and Tao (4) have used blood protected against coagulation by the addition of sodium citrate. Various workers have studied the course of pneumococcus infection in the blood stream of experimental animals: Wright, H. D. (5), Goodner, K. (6, 7), and Cecil and Blake (8, 9, 10, 11, 12, 13). The most recent work on phagocytic immunity is that of Robertson and his co-workers (14, 15, 16) with serum-leucocyte mixtures. The methods described below are in part adaptations of those used by these last-named investigators. In the early part of this study serum-leucocyte mixtures were tried repeatedly, but the manipulations required to free the leucocytes from the whole blood were, in our experience, too numerous and delicate for practical use. It occurred to us that whole uncoagulated blood might possibly present similar immunological features and that if this were so, it could be substituted with advantage for the serum-leucocyte mixtures.

MATERIALS AND METHODS

An agitator was constructed as illustrated in figure 1. The motive power of the agitator is a 4-volt storage battery. Speed is regulated by a rheostat starting key

¹ This work also received financial aid from the Research Fund of the Metropolitan Life Insurance Company.

at R. The eccentric wheel, on the circumference of which the tubes are placed, provides rotation and end to end mixing of the contents of the tubes.

Heparin (Howell, 17, 18), was used as the anticoagulant. Mechanical defibrination changes the white blood cells by destroying or removing many of the polynuclear cells as shown by Bull (20). Potassium or lithium oxalate in concentration necessary for preventing coagulation has some bacteriostatic action in cultures of pneumococci. Heparin in the concentration used in the blood in this study has no apparent effect on the growth of the pneumococcus.

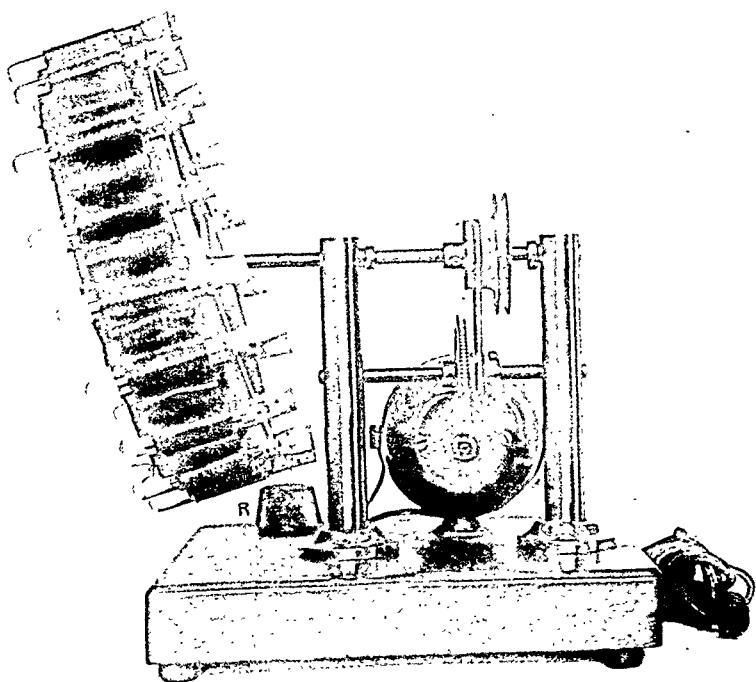


FIG. 1. ECCENTRIC ROTATOR TO MIX TUBE CONTENTS

Since the completion of this work the anti-complementary action of heparin has been studied by Ecker and Gross (19). We have been able to confirm this action of heparin when added directly to serum and also when used as an anticoagulant in whole blood. The amounts of heparin needed to demonstrate anti-complementary power are, however, greater than any that were used in this study. We feel it safe to say that in the whole blood samples used in this study, the plasma would in all probability have been active in the usual sheep cell and antigen system.

The test was performed as follows:

The blood sample to be tested was collected under sterile precautions, in 5 to

15 cc. amounts in a pyrex flask containing 1 mgm. of heparin in 0.1 cc. of normal saline for each 5 cc. of blood. The flask was kept in a dish of cracked ice before receiving the blood and from 5 to 10 minutes thereafter. A total white cell count and a differential count were made on the blood in the flask after it was received in the laboratory.

Three cubic centimeters of an 18 hour broth culture of Type I pneumococci of a virulence sufficient to kill 20 gram mice regularly in a dilution of 10^{-8} were seeded in 25 cc. of sterile broth in a 50 cc. centrifuge tube. This culture became dense enough in 3 to 4 hours to appear opaque at the greatest diameter of the tube ($1\frac{1}{2}$ inches). It was centrifugalized at high speed for 15 minutes, the supernatant broth poured off, and the organisms taken up in 5 cc. of gelatin-Locke's solution. The density of this suspension was read by the Gates method (21) and diluted

TABLE 1
Contents of tubes when assembled

Tube number	Blood	Pneumococci	Obtained from 0.1 cc. of
	<i>cc.</i>		
1	0.5	10^8	Standard suspension
2	0.5	10^7	Dilution 1
3	0.5	10^6	Dilution 2
4	0.5	10^5	Dilution 3
5	0.5	10^4	Dilution 4
6	0.5	1,000	Dilution 5
7	0.5	100	Dilution 6
8	0.5	100	Dilution 6
9	0.5	0	Gelatin—Locke's solution

to a standard density equivalent to approximately 1000 million pairs of pneumococci per cubic centimeter. The reading for this density with the Gates nephelometer was 1.1 cm. for the work to be reported.

Dilutions of the standard suspension of pneumococci were made in gelatin-Locke's solution. Each dilution was 1:10 of the preceding dilution.

The culture dilutions were now placed in the 9 small tubes, as indicated in table 1. A 1 cc. pipette with tip about 8 cm. long was used to place the culture well in the bottoms of the tubes. One tenth cubic centimeter of dilutions 4, 5, 6, and 7 were plated in blood agar as a check on the number and viability of the pneumococci actually placed in a test.

The blood was then added. It was mixed by gentle rotation of the flask. Five tenth cubic centimeter amounts were introduced to the bottom of all the tubes, except the 8th, with a 2 cc. pipette drawn out at the tip. The 8th tube received 0.5 cc. of plasma obtained from the heparinized blood by centrifugalization.

The contents of the 9 tubes, when the test was completely assembled, are illustrated in table 1.

The corks were then dipped in melted paraffin, cooled and placed in the tubes, after flaming the lips of the tubes slightly.

The tubes were inserted in the leather band on the eccentric wheel of the agitator and the time noted. The rheostat was adjusted so that the wheel revolved at the rate of 15 to 25 revolutions per minute. Agitation was continued for 6 hours; then the tubes were set upright in a rack and replaced in the incubator for 18 hours.

In reading the results of the test, the color change was recorded, the appearance of the growth in the plasma control tube noted as to density and uniformity, and pour-plate cultures were made from each of the tubes in the test. It has been our experience from repeated estimations and dilutions of such cultures that a plate that appears uniformly brown, indicated in protocols by the symbol " α ," contains more than 5000 colonies. Any less than 5000 pneumococci can be estimated approximately and this estimation has been indicated by the symbol " α -", meaning a lesser degree of growth than a "brown" plate, but still too great a number of colonies to be counted.

We have used the standard procedure for the mouse protection test as described in a number of text books.

Consistency of the measurements

Various checks were employed for testing the accuracy of the methods used for counting and distributing the pneumococci and white cells in the tubes. The system of standardizing and diluting the suspension of organisms was analyzed by making pour-plate cultures in blood agar with every performance of the test. Granting that the standardized suspension contained 1000 million pairs of pneumococci per cubic centimeter, and that the dilution of each succeeding tube by one tenth was perfectly carried out, 100 colonies should result from planting 0.1 cc. of the 6th dilution. Dilutions 5 and 7 should contain 1000 and 10 organisms, respectively, in each 0.1 cc. As a matter of record, the variation in the number of colonies obtained by planting 0.1 cc. of the 6th dilution in 15 consecutive tests was from 35 to 172. The average number of colonies was 93. The extreme variation between tube 6 and tube 7 was 72 per cent of the theoretical difference between the dilution in tubes 6 and 7, but between tubes 5 and 6 the extreme variation was only 8 per cent.

Viability of the leucocytes

The presence and duration of life in the leucocytes under the conditions of the test were determined, roughly, by the use of unstained

warm stage preparations. Blood to which pneumococci had been added was examined, as well as blood to which no pneumococci had been added, but which, in other respects, had been subjected to the same manipulations (tube 9). At the end of 6 hours rotation and 24 hours incubation the polynuclear leucocytes in the blood without pneumococci presented some changes. They did not appear as active as when first examined and the cytoplasm contained a moderate number of green refractive vacuoles. Observations on the same blood were made at intervals up to and including 72 hours. The polynuclear cells became more and more sluggish during that time; many lost the power of motility entirely, while others retained it to a slight degree. The

TABLE 2
Changes in the reading of the test after the end of the 24-hour period

Amount of culture suspension	24 hours		72 hours
	Color change	Number of organisms	Color change
0.001	+	∞	++++
0.0001	0*	∞	++++
0.000075	0	4,500	++++
0.00005	0	3,000	++++
0.000025	0	7	++++
0.00001	0	0	0

*0, no change; +, slight change; ++, darker red; +++, deep red; +++++, brown.

appearance of the lymphocytes was the same throughout, although they became motile toward the end of 72 hours incubation.

In the presence of actively growing pneumococci the changes mentioned above occurred much more quickly. The polynuclears were reduced in numbers and the remaining cells were filled with green vacuoles within six hours agitation and incubation. Practically all the polynuclears were destroyed within 18 to 24 hours incubation. Heist and his co-workers (2) made similar observations on the destruction of cells and fibrin by the pneumococci. They described it as apparent digestion by the pneumococci.

The less active the growth of the pneumococci the smaller the degree of destruction of the polynuclears. Where no growth occurred, that is, in blood which killed all the pneumococci which had been added to

it, the polynuclears presented no changes in 24 hours in either motility or in the appearance of the cytoplasm, other than those changes observed in the control blood to which sterile gelatin-Locke's had been added.

The lymphocytes, as judged by the observation of warm-stage preparations and counts, were not affected by the growth of pneumococci in the blood. A number of unidentified cells developed in preparations with and without pneumococci.

End point of the test

Robertson and Sia made the final readings of their tests at the end of 72 hours, while other authors observed their results at the end of 18 or 24 hours. Several of our tests were read at both 24 and 48 hours, and a few were read at the end of 72 hours. The main difference in these readings is indicated in table 2. At the end of 72 hours there was no question about the color change which appeared in all tubes in which pneumococci remained viable. In 24 hours the color change caused by methemoglobin formation was not observed in some tubes in which many pneumococci were living and multiplying, but cultures at the end of 24 hours indicated all the tubes in which growth could continue.

For a time, blood broth cultures, blood agar plate cultures, mouse injection, and microscopic examination of the tube contents were used as indicators of the survival or death of the pneumococci. In regard to the first two, it was found that broth cultures were sometimes positive when plate cultures were negative and vice versa, but the difference was, at most, a matter of a very few organisms.

The results of mouse inoculations agreed with those of broth or plate cultures only when no serum protection for mice was present. The examination of stained smears could be used to indicate small numbers of organisms, as well as large numbers, if sufficient care was taken in the search.

The greater accuracy of blood agar plates, however, and the knowledge that the colonies indicated viable organisms seemed to us marked advantages.

Two controls are indicated in the diagram of the test table 1 as tubes 8 and 9. Tube 8 contained plasma from heparinized blood

seeded with the smallest number of pneumococci used in the test, namely, an average of 100 pairs. By this means any influence of the blood plasma upon the pneumococcus was found, such as agglutination, judged macroscopically. Tube 9 contained blood with gelatin-Locke's solution, but no pneumococcus suspension. Growth in this control indicated that the patient's blood already contained organisms capable of proliferation. During approximately one-half of the work a third control, seeded with 100 pneumococci, was used, namely, red blood cells and plasma from which nearly all the white blood cells had been removed by centrifugalization. Growth in this control throughout our experiment was the same as in the plasma alone, and finally the use of this control was discontinued.

Results of repeated tests on the same subjects

A consideration of prime importance in the use of this test was a measure of its consistency when applied to the blood of one normal individual several different times. Two tests were made on the blood of five different individuals. In three of these comparisons agreement was perfect. In the two remaining comparisons there was a difference amounting to that between one tube and the next.

In further tests we had occasion to repeat the estimation of the pneumococcal power of the blood of a laboratory worker who had no history of pneumococcus infection, but whose serum had some protection for the mouse. This test was done on 8 different days, with a total of 10 estimations. In only one instance was there any disagreement and that was to the same degree as above, namely, one tube. Since the method of making the culture dilution might account for this much variation, we felt that the results were satisfactory.

Whole blood immunity in patients with no history of pneumonia

Patients who gave no history of lobar pneumonia but who had been admitted for chronic or minor ailments were chosen for this part of the study. None of them had febrile infections at the time of the test. The diagnoses were dyspepsia, adhesions of the stomach, peptic ulcer, carcinoma, chorea, chronic cardiac valvular disease of rheumatic origin, chronic arthritis, chronic gonorrheal infection of the joints and urethra, acute nephritis, hypertension, arteriosclerosis, senility, neurasthenia, and urticaria.

The number of pneumococci killed by 0.5 cc. of uncoagulated blood was determined for 27 different individuals. The results are given in table 3. The blood from 9 of the subjects killed no pneumococci; the blood from 17 killed from 100 organisms to 10,000 organisms, while the blood from one subject killed 100,000 organisms. Thus in hospital patients with no history of pneumonia, the pneumococidal power of 0.5 cc. of whole blood for pneumococcus Type I varied from nil in one third of the subjects to 100 to 10,000 organisms in two thirds of the subjects, with one subject whose blood killed an even greater number.

TABLE 3

Pneumococidal power of whole blood and protective power of serum in hospital patients with no history of pneumonia

Mouse protection in serum M.L.D.	Pneumococidal power of whole blood (number of organisms killed)					
	0	100	10 ³	10 ⁴	10 ⁵	10 ⁶
0	3	1	5	4	0	0
1	0	1	1	1	0	0
10	0	0	1	1	0	0
100	0	0	0	0	1	0
10 ³	0	0	0	0	0	0
10 ⁴	0	0	0	0	0	0
Protection test not performed	6	1	1	0	0	0
Total.....	9	3	8	6	1	0

Reading from the left in the same table the results of the mouse protection tests are expressed as the number of minimal lethal doses (M.L.D.) of pneumococcus culture against which 0.2 cc. of the serum is effective in saving the life of a mouse. The serum for these tests was obtained from the subjects at the same time the whole blood sample was taken. The serum from 19 of the subjects was examined for protective power. Thirteen of the subjects gave no evidence of protection, while 6 had protective power against a small amount of culture. The measure of protection in normal individuals has been reported by Cecil and Austin (22) and by Clough (23, 24) with similar results.

The degree of pneumococidal power can be compared to the degree

of serum protection for the mouse in these 19 subjects. In the first place, while two thirds of the subjects had some whole blood pneumococidal power, only one third had serum protection for mice.

To a certain extent, however, there is a relation between serum protection and whole blood immunity, inasmuch as among the six patients who had serum protection, all had some pneumococidal power. The subject with the highest degree of protection (against 100 M.L.D.) also had the highest degree of whole blood pneumococidal power (100,000 organisms). There is, however, a group of 13 subjects without any serum protection, 10 of whom had some whole blood pneumococidal power. The degree of pneumococidal power in the 10 subjects without serum protection ranged from 100 to 10,000 organisms, which includes the degrees found most frequently in the whole group of normal subjects.

These hospital patients with no history of lobar pneumonia were studied in lieu of strictly normal individuals to provide a base line with which to compare the degree of pneumococidal power found in patients with pneumonia. We are encouraged to regard this base line as significant by the fact that this group of subjects presented measurements, in serum protection for mice, which were similar to measurements for groups of strictly normal individuals studied by other workers.

SUMMARY

1. A method is described for measuring the capacity of 0.5 cc. of uncoagulated human blood to kill pneumococci.

2. The blood of 17 out of 27 hospital patients who had not had lobar pneumonia killed from 100 to 10,000 virulent Type I pneumococci. The blood of 9 of the same group of patients killed no pneumococci.

3. When both mouse protection and whole blood pneumococidal power were measured, 6 subjects had both, 10 subjects had whole blood pneumococidal power alone and 3 subjects had neither whole blood pneumococidal power nor mouse protection.

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THE PNEUMOCOCCIDAL POWER OF WHOLE BLOOD

II. ESTIMATIONS IN LOBAR PNEUMONIA¹

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Our primary purpose in using the whole blood technique described in a previous communication (1) for measuring pneumococcidal power was its application to the study of lobar pneumonia in patients with the disease. Seventy-six separate observations have been made on 35 different patients. These observations have been grouped according to their relation to the stage of the disease, as follows: (a) before the crisis, (b) at the crisis or later, (c) with complications and (d) about the time of death. In two cases the changes were compared with the findings of other serological reactions at various stages of the disease. The whole blood immunity, as affected by the administration of serum, was also studied in a few patients. All patients observed were suffering from lobar pneumonia caused by the Type I pneumococcus, unless otherwise stated.

The results of our tests with blood, the serum of which was highly mouse protective, differ considerably from the results obtained when blood from individuals with no history of pneumonia and with no serum protection for mice was tested.

The protocols of two typical tests are given in table 1. In the first protocol is shown the pneumococcidal power of the blood of a person who had no history of pneumonia and who had no serum immunity detectable by the usual mouse protection test. The blood of this subject killed 1000 pneumococci. The blood agar plate planted with

¹ This work also received financial aid from the Research Fund of the Metropolitan Life Insurance Company.

0.1 cc. of the contents of the first tube in which growth occurred, (number 5), became brown, that is, it contained an uncountable number of pneumococcus colonies. In the second protocol the pneumococidal power of an immune blood is shown. The immune blood killed 100,000 organisms. In two tubes in which actual sterilization did not take place there was a reduction in the number of organism originally added to the blood. This was shown by the subsequent growth in the blood-agar plates from tubes 2 and 3 of less than 5000

TABLE 1
Specimen protocols

1. Blood from patient with no history of pneumococcus infection. 2. Blood from patient convalescing from lobar pneumonia due to Type I pneumococcus.

Tube number	Number of organisms	Protocol 1. Non-immune blood			Protocol 2. Immune blood		
		Color change	Culture		Color change	Culture	
			Amount	Growth		Amount	Growth
1	10 ⁸		cc.		+++	0.1	∞
2	10 ⁷	+++	0.1	∞	0	0.6	∞ —
3	10 ⁶	++	0.1	∞	0	0.6	1,250 colonies
4	10 ⁵	0	0.6	∞	0	0.6	0
5	10 ⁴	0	0.6	∞	0	0.6	0
6	1,000	0	0.6	0	0	0.6	0
7	100	0	0.6	0	0	0.6	0
8	100	Diffuse growth	0.1	∞	Diffuse growth	0.1	∞
9	(plasma) 0	0	0.6	0	0	0.6	0

pneumococcus colonies when 100 million and 10 million organisms had originally been introduced into the tubes. Such an inhibition of the growth of pneumococci was produced only by the blood of individuals who had a degree of serum immunity easily demonstrable by the mouse protection test.

WHOLE BLOOD IMMUNITY DURING THE ACUTE PHASE OF LOBAR PNEUMONIA

During the febrile stages, before the crisis of lobar pneumonia in patients who are destined to recover, it is likely that the immune

mechanism is actively at work. Ten blood samples were examined from 7 different subjects in this active stage of Type I lobar pneumonia. None of these patients received any antipneumococcus serum or any other specific treatment. Although the first patient to be considered below finally died with erysipelas, he is included in this series because he passed through a distinct crisis and appeared to recover from the acute phase of lobar pneumonia. The 6 other patients made complete and permanent recoveries.

The blood specimens examined both for pneumococcal power and serum protection will be taken up in the order of their relationship to the day of crisis. The description of "the day of crisis" that has been adhered to is as follows: a day on which the rectal temperature reached 101°F., and following which a marked improvement in the condition of the patient was manifest.

Our earliest observation during the course of lobar pneumonia was made on the second day of the disease, five days before the crisis. The findings throughout this case are illustrated in figure 2. The blood cultures taken at the bedside yielded 12 Type I pneumococcus colonies per cubic centimeter. Growth occurred in the heparinized blood, even when no pneumococci were added.

The next observations in order of their approach to the crisis are those on three cases made three days before the drop of temperature to 101°F. In these cases the pneumococcal power showed wide variations. In one case, in which the blood culture was weakly positive, no pneumococcal power was present, though no spontaneous growth occurred in 0.5 cc. of blood to which no pneumococci were added. Of the two other patients, both of whom had negative blood cultures, blood from one killed 10,000 pneumococci, and blood from the other killed 1,000,000. Neither of these patients showed any serum immunity when tested by the routine protection test in mice.

Two observations were made on the second day before the crisis. Both of these patients had positive blood cultures, and both showed whole blood immunity for the Type I pneumococcus. In the first case, examined two days before the termination of the acute phase of the disease, the blood culture was weakly positive, 5 cc. of blood being required to produce growth. In this case the blood sample sterilized itself and killed 10,000 pneumococci, while the serum sample showed a

small amount of protection. This protection test was done after the serum had been kept in the ice box for a period of 20 days. In the second of these observations which was made on the 5th day of the disease (fig. 2) the blood culture showed 4 pneumococci per cubic centimeter of blood. The blood sample with heparin added sterilized itself, and in addition killed all pneumococci in the tube seeded with 1,000,000. No serum protection was found in this specimen when the test was done one month later. This test, as well as others, appears to indicate some independence of the whole blood immunity against the pneumococcus from serum protection for the mouse.

In the three cases examined for pneumococcidal power on the day before crisis, the blood itself was sterile, and the pneumococcidal power was of a high degree. Blood from one case killed 10,000 pneumococci, from another 1,000,000 pneumococci, and from the third 10,000,000 pneumococci. Tests with serum in the mouse likewise showed a considerable amount of protective power in each case.

These ten tests, made during the acute stage of Type I pneumonia in patients that ultimately recovered, may not include all possible combinations of immunological factors. They bring out, however, several points that may be summarized as follows: 1. The multiplication of organisms, already present in the patient's blood stream, after the blood has been withdrawn and subjected to the conditions of the test, is compatible with the patient's recovery from the acute stage of the disease. 2. The power of the blood to kill pneumococci introduced into it may develop (*a*) in the presence of bacteremia, and (*b*) independently of the serum antibodies as revealed by the routine mouse protection test.

WHOLE BLOOD IMMUNITY AT THE CRISIS AND AFTER THE CRISIS

The highest level in the development of whole blood immunity was reached the day before the actual fall in temperature to 101° and was maintained at that level for one week after the crisis. A total of 26 observations was made on 13 patients both on the day of the crisis and at subsequent intervals up to the 28th day after crisis. The thirteen tests made in the first week after crisis all showed a high degree of pneumococcidal power. In eight of these tests 100,000 organisms were killed by 0.5 cc. of blood and in five 1,000,000 organisms were

killed. All these bloods showed serum protection for mice and marked inhibitory power in the tubes in which complete sterilization did not take place.

WHOLE BLOOD IMMUNITY IN THE PRESENCE OF PNEUMOCOCCUS COMPLICATIONS

Blood samples from three patients suffering from complications due to the pneumococcus were measured for their pneumococcal power. In each case the whole blood immunity was as high as that found after the crisis in uncomplicated cases of lobar pneumonia. In the first case, illustrated in figure 1, the development of whole blood immunity and serum protection for mice had been observed previous to the detection of empyema on the 12th day after the crisis. The blood culture was negative when empyema was recognized, and the change in whole blood immunity from the level previously established was slight. Ten thousand organisms were killed and considerable inhibition of growth occurred in tubes planted with as many as 100,000,000 organisms. Serum protection for mice was lower the day following the detection of empyema than it had been 4 days previously. The fall in protective power was from 100,000 M.L.D. to 1000 M.L.D. This is not an unusual change in uncomplicated convalescence from lobar pneumonia and may have little significance. In the second case the complication was arthritis, due to a Type II pneumococcus. Eight days after the detection of the purulent accumulation in the right shoulder joint and 29 days after the onset of pneumonia the pneumococcal power was unusually high, 1,000,000 Type II pneumococci being killed. Inhibition occurred in the tube inoculated with 10,000,000 organisms. The blood culture was positive only in the flasks seeded with 5 cc. of blood. The blood serum contained no Type II protection for mice. The third case was a pneumococcus Type I endocarditis. This patient had received, during the 7th, 8th, 9th and 10th day of his disease, 195 cc. of concentrated antipneumococcus serum containing 390,000 units of protection. Pneumococcus endocarditis was diagnosed by means of blood cultures, the development of an aortic diastolic murmur, and petechiae which appeared on the 30th day of his illness. The degree of whole blood immunity was high. One hundred thousand Type I pneumococci were killed, and in the

tube inoculated with 10,000,000 organisms less than 5000 survived. One cubic centimeter of the patient's blood cultured in agar yielded 110 Type I pneumococcus colonies. The serum of a sample of blood obtained simultaneously with the samples for blood culture and whole blood immunity test protected a mouse against 100,000 M.L.D. of Type I pneumococci. This protection test was done 41 days after the blood was drawn.

WHOLE BLOOD IMMUNITY ABOUT THE TIME OF DEATH

A certain relation has been observed between the presence of pneumococci in the blood stream and a fatal termination in lobar pneumonia. It seemed desirable, therefore, to determine the degree of bacteremia and the pneumococcidal power of the blood in moribund patients. The blood of three patients each suffering from Type I pneumococcus pneumonia was examined, one 3 hours, one 12 hours, and one 15 hours before death took place. All three patients appeared moribund at the time the blood was taken for examination, and all three had positive blood cultures. There was no evidence of any whole blood immunity in two of the patients whose blood *in vivo* contained 84 and 304 colonies of pneumococci per cubic centimeter respectively; indeed, the blood became overgrown by the Type I pneumococci already present without further inoculation of organisms. In the case of the third patient, whose blood contained 42 Type I pneumococci per cubic centimeter three hours before death, the whole uncoagulated blood killed not only those present but also killed 10,000 additional Type I pneumococci.

RELATION OF IMMUNE REACTIONS TO CLINICAL COURSE

In two cases of Type I pneumonia observations were made several times during the course of the disease. The accompanying charts show the temperature, the white blood count, the number of organisms in the blood, the whole blood immunity, the agglutination qualitatively observed, and the mouse protection test.

The patient represented in figure 1 was a man 36 years of age, who entered the hospital 31 hours after the onset of lobar pneumonia. His first blood specimen was obtained on the third day of his disease, the first day charted in the figure. His temperature fell sharply to

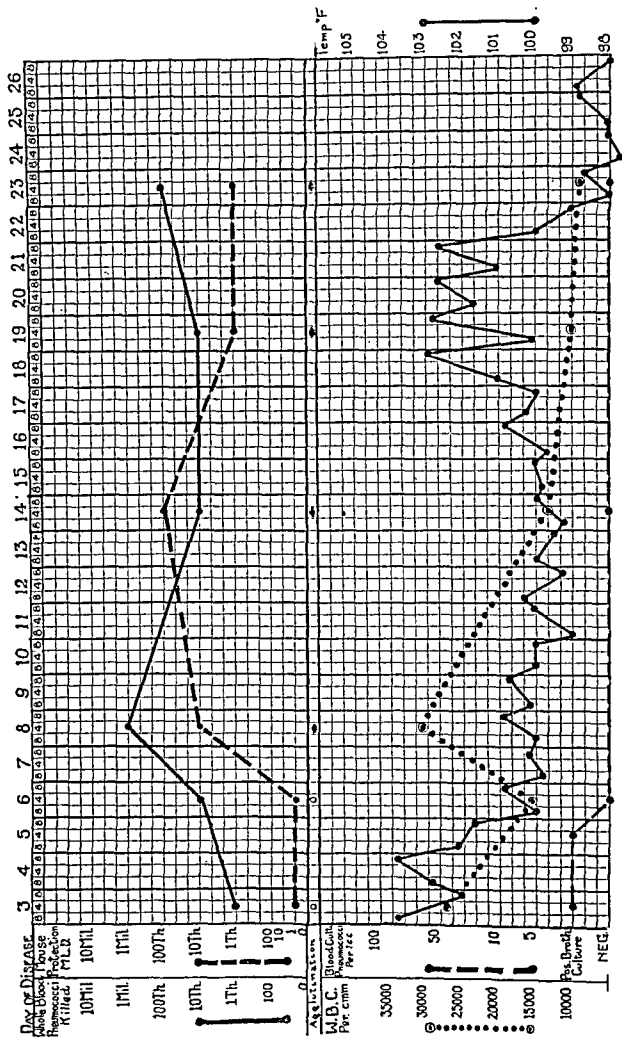


FIG. 1. OBSERVATIONS DURING THE COURSE OF A CASE OF TYPE I LOBAR PNEUMONIA FOLLOWED BY EMPYEMA.
RECOVERY

100° on the 6th and 7th day, but rose again from the 14th to the 18th days. This was due to the development of empyema for which he underwent operation the 21st day of his illness. Recovery followed soon after thoracotomy was performed. This patient had a small amount of protection in his serum on the third day of his disease,

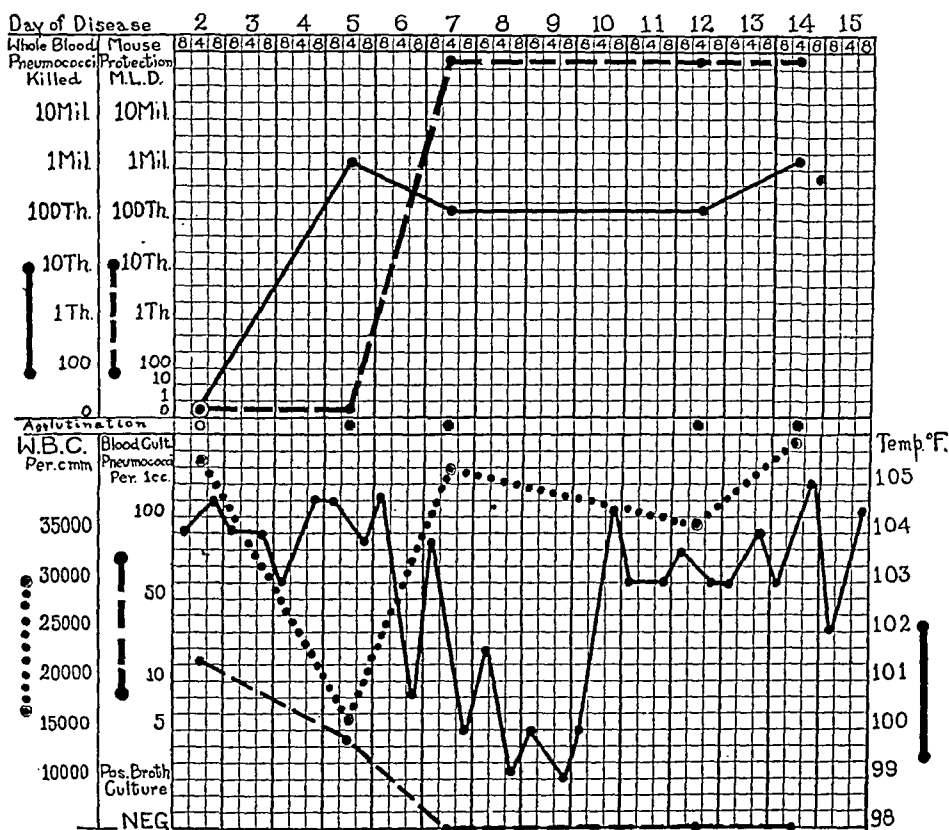


FIG. 2. OBSERVATIONS DURING THE COURSE OF A CASE OF TYPE I LOBAR PNEUMONIA FOLLOWED BY EMPYEMA AND ERYSIPELAS. FATAL TERMINATION

together with some pneumococcidal power, but showed simultaneously a positive blood culture in broth inoculated with 5 cc. of blood. An increase of pneumococcidal power was evident in the first specimen in which the blood culture was negative. Both pneumococcidal power and protective power reached a high point on the day of the sharp drop

in temperature. Agglutination appeared in the undiluted plasma on the day of the crisis.

Figure 2 represents the chart of a young man 28 years of age who entered the hospital on the first day of his disease. The first blood sample was obtained on the second day of his illness which is the first day shown on the chart. His temperature fell sharply to normal on the 7th day, rose again to 102°F., and then fell to 99°F. on the 8th day. On the tenth day it rose again and remained high until death on the 16th day. On the 13th day erysipelas appeared on the face. The patient died and at autopsy empyema was found in the left thorax. The pneumococcal power of the blood appeared before the serum protection for mice. Agglutination appeared simultaneously with the appearance of protective power for mice. This patient, when first seen, differed from the preceding one in that he showed a more heavily infected blood culture. On the second day of his disease agar plates showed 12 Type I pneumococcus colonies per cubic centimeter of blood. In other cases, even shortly before death, a still greater number of organisms had been disposed of by the blood itself, but in this instance the organisms already present multiplied vigorously. At the second test made three days later, on the 5th day of the disease, there were 4 organisms per cubic centimeter in the patient's blood stream but these were killed and sterilization took place in tubes inoculated with as many as 1,000,000 Type I pneumococci. Serum protection for mice did not develop at this time, but was present on the 7th day, which was the day before crisis.

EFFECT OF SERUM ADMINISTRATION UPON WHOLE BLOOD IMMUNITY

It is of interest to note the effect of specific treatment on the ability of the blood to kill pneumococci. A patient with Type I pneumonia, on the fourth day of the disease was selected for the test (fig. 3). His blood contained enough organisms to give a positive culture when 5 cc. were inoculated into 50 cc. of broth. Before the administration of serum there was a slight amount of pneumococcal power, sufficient to kill 100 pneumococci, in addition to those already present in the patient's blood stream. After the administration of 80 cc. of concentrated antipneumococcus serum containing 160,000 units of mouse

protection, the blood culture was sterile and whole blood pneumococidal power had increased to such a degree that 1,000,000 pneumococci were killed. Protective power in the patient's serum and agglutination appeared simultaneously. Agglutination was still present the fourteenth day after the onset of the disease. The protective

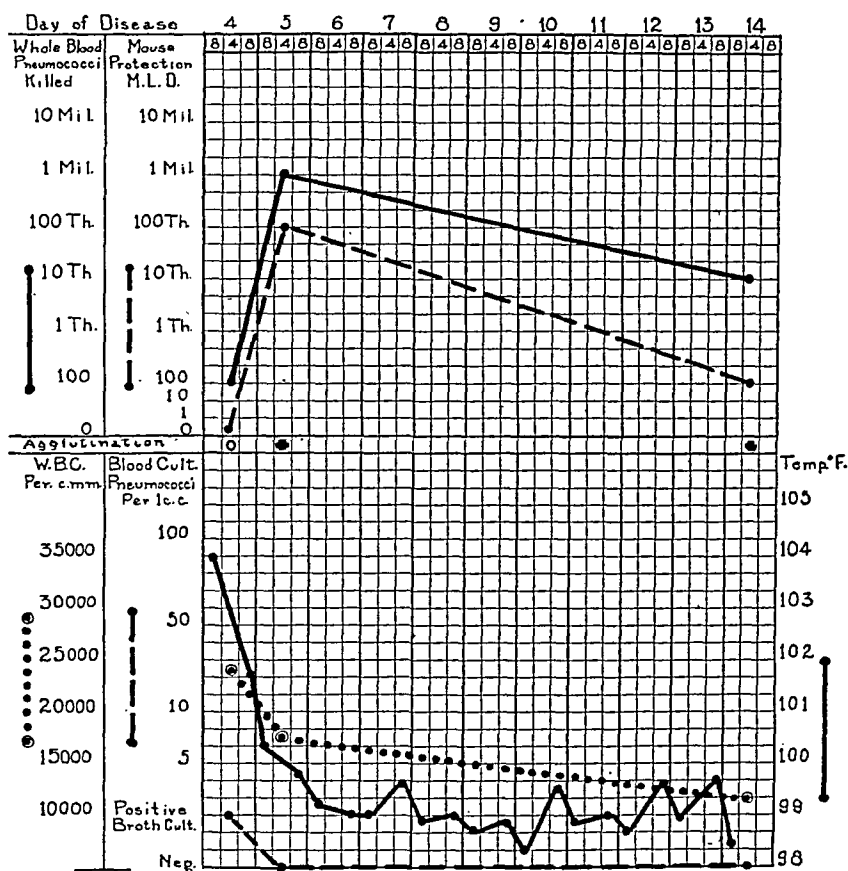


FIG. 3. OBSERVATIONS DURING THE COURSE OF A CASE OF TREATED TYPE I LOBAR PNEUMONIA WITH FAVORABLE OUTCOME

power for mice appeared to fall off more quickly than did the whole blood immunity.

DISCUSSION

This series of tests was designed to study the gross features of the whole blood immunity of man against the pneumococcus. The data

obtained does not bear directly on the partition of the pneumococidal power of the whole blood between the humoral and cellular portions of the blood. A survey of the data discloses nothing inconsistent with the position taken by Robertson and Sia with respect to the immunity of animals (2) and man (3). Throughout our study in man serum immunity and whole blood immunity have been roughly parallel in the individual case.

The leucocyte is necessary for the reaction but, if the normal number is present, further increase has little or no effect upon the number of pneumococci which either the serum-leucocyte mixture or whole uncoagulated blood can kill. This point was brought out by Sia, Robertson and Woo (4) and may also be illustrated by our experiments on man. When an infection is at its height, such as before the crisis in lobar pneumonia, or in the presence of general septicemia, the white cell count is usually well above normal, with a high percentage of polymorphonuclear leucocytes. In the cases in this study that was frequently the time at which pneumococidal power was low. With the drop in the count to normal the pneumococidal power reached and maintained its highest level. In one test the maximum killing power, that effective against 1,000,000 pneumococci, appeared in the presence of 2,080,000 polynuclear leucocytes (4,160 per cubic millimeter), and, in another, in the presence of 11,800,000 polynuclear leucocytes (23,600 per cubic millimeter), or about five times as many. On the other hand, no pneumococidal power was found in samples of blood which contained, at one extreme, 17,200,000 polynuclear leucocytes (34,400 per cubic millimeter) and at the other extreme 4,210,000 polynuclear leucocytes (8,420 per cubic millimeter).

Since certain degrees of whole blood immunity occur in normal subjects independently of serum protection, as detected by the usual mouse protection test, we should not expect the whole blood immunity in disease to follow exactly the serum protection for mice. In general, an increased power of the whole blood to kill pneumococci develops earlier in the acute stage of the disease than does protection for mice. Aside from this finding, however, serum immunity, as measured by the mouse protection test, and whole blood immunity run fairly parallel. The difference between serum-protection and whole blood immunity may be due to a greater sensitiveness of the whole blood test.

Many writers have emphasized the importance of bacteremia in lobar pneumonia from the standpoint of prognosis and treatment. The presence of more than a certain number of organisms, especially later than the 5th day of the disease, is a fairly trustworthy indication of a fatal outcome. On the other hand, the presence of a few pneumococci in the blood in the early stages of the disease has little prognostic significance. A comparison between the findings of the whole blood test and the clinical course of the disease may give some idea of the significance of pneumococci in the blood and their relationship to the outcome in lobar pneumonia. On the whole, patients whose blood, when drawn, can kill whatever pneumococci are present therein, are doing well and those patients whose blood becomes overgrown by the organisms already present in the blood stream are doing badly. There are some conspicuous exceptions, however, to this general rule. On the one hand, the blood of one patient failed early in the disease to become sterile under the conditions of the test. Just before the crisis the blood of this patient developed the ability to sterilize itself and to kill a large additional number of pneumococci. On the other hand, we have observed a patient's blood that contained a number of organisms only three hours before death, which did sterilize itself. A special instance of the relationship of the pneumococcidal power of the blood to the outcome of the disease is manifested in pneumococcus complications. In the cases examined in this study (empyema, purulent arthritis, and endocarditis), the blood retained its ability to kill pneumococci present in the blood stream, and also the ability to kill a large number of pneumococci inoculated after the withdrawal of the blood. Although infection of the blood is an important symptom in the course of lobar pneumonia, it is apparently not the only factor associated with death, nor is it necessarily followed by death.

The actual mechanism of recovery or death does not seem to us adequately explained by the results of tests of blood or serum against pneumococci *in vitro*. In the 35 cases above, the whole blood immunity serum immunity, and the outcome of the disease are roughly parallel, except in the presence of purulent complications. Yet the correlation is not strict. Bacteria penetrate into the blood in the presence of bactericidal power death occurs in the presence of few or many bacteria.

and occasionally in the presence of a certain amount of bactericidal power. The mechanism of recovery or crisis is difficult to understand in view of the difference in time between the appearance of the blood immune phenomena and the improvement in the condition of the patient. The missing correlation may lie, however, not in the lack of cause and effect, but in the lack of an exact knowledge of the mode by which the bacteria or their products produce death, or the immune products, on the other hand, produce crisis or recovery.

SUMMARY

1. The blood of 7 patients acutely ill from Type I lobar pneumonia killed from no pneumococci to 10,000 pneumococci in 6 observations made on the 1st, 2nd, 3rd, 4th and 5th days before the crisis. Four blood samples taken on the 1st, 2nd, and 3rd days before the crisis killed from 100,000 to 1,000,000 Type I pneumococci.

2. Thirteen blood samples examined during the first week after the crisis killed from 100,000 to 1,000,000 pneumococci.

3. Thirteen blood samples examined during the 2nd, 3rd and 4th week after the crisis killed from 1000 to 100,000 pneumococci.

4. Before the crisis, bacteremia was found to be associated with great variations in the pneumococcidal power of the blood; from no pneumococci to 1,000,000 pneumococci were killed. Bacteremia or sepsis about the time of death was usually associated with no pneumococcidal power, but in one case out of three, a moderate degree of pneumococcidal power and self-sterilizing power were present.

5. In the presence of pneumococcus complications such as empyema, purulent arthritis, and endocarditis, the patients' blood often contained pneumococci and was at the same time markedly pneumococcidal.

6. The administration of concentrated antipneumococcus serum to patients with lobar pneumonia produced a sudden rise in the pneumococcidal power of the blood to that level which is found in patients who have successfully passed the crisis.

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RÉSUMÉ OF THE LITERATURE

Pulse rate and pulse pressure. In general, investigators have found that there is a definite, though inexact, correspondence between the elevation in the basal metabolic rate and the increase in pulse rate. Sturgis and Tompkins (2), in a study of 154 patients with thyrotoxicosis, found a fairly constant relationship between the resting pulse rate and the basal metabolism. They state that "a basal pulse rate below 90 per minute is seldom, and below 80 per minute is rarely associated with an increased metabolism." The rise in pulse rate is due, presumably, to increased metabolism for Minot and Means (3) observed that the degree of pulse elevation for a given metabolic rate was essentially the same in thyrotoxicosis and in chronic leukemia. Read (4), and Davies and Eason (5) corroborated the observations of Sturgis and Tompkins, finding, in spite of numerous exceptions, a general relation between the pulse rate and the basal metabolic rate. Davies and Eason also observed that as the basal metabolic rate increased, the pulse pressure likewise tended to increase. An increased pulse rate usually signifies an increased blood flow although blood flow may be actually diminished if a great reduction in the stroke volume occurs (6, 7). In brief, the pulse rate and the pulse pressure tend to be elevated with increase in minute volume output, but the relation is a varying one.

Vital capacity of the lungs. Rabinowitch (8) studied the vital capacity of the lungs in a series of patients with thyrotoxicosis and observed that it became lower as the basal metabolic rate increased. McKinlay (9) likewise found a reduction in the vital capacity of the lungs to below 70 per cent of the normal in a great majority of severely toxic cases of thyroid disease. He observed that the minute volume of pulmonary ventilation at rest was not related to the diminution in the vital capacity of the lungs. Lemon and Moersch (10) compared the vital capacity of the lungs and basal metabolic rate in 85 subjects. They found a tendency toward decreased vital capacity with increased metabolic rates, but stated that there was no precise relationship between the two measurements in a given individual.

Minute volume output of the heart. Plesch (11) studied the minute volume output of the heart in one case of exophthalmic goitre, using an ingenious but rather crude gasometric method. He found that the minute volume output of the heart was 5,288 cc. as contrasted with 4,359 cc. in one normal subject. The pulse rate of the patient with exophthalmic goitre averaged 97, that of the normal subject 72 per minute, while the oxygen consumption was 6.47 cc. per kilo per minute as compared to 3.52 cc. for the normal subject. Rabinowitch and Bazin (12) studied the venous oxygen unsaturation of the arm blood in patients with thyrotoxicosis, and inferred that no significant increase occurred in the minute volume output of the heart or in the output per beat. Liljestrand and Stenström (13) carefully studied the minute volume output of the heart in ten healthy subjects and in eleven patients with exophthalmic goitre, using the nitrous oxide method of Krogh and Lindhard (30). Eight female patients with an average increase in the

STUDIES ON THE VELOCITY OF BLOOD FLOW

XIII. THE CIRCULATORY RESPONSE TO THYROTOXICOSIS¹

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Parry in 1815 (1) originally described exophthalmic goitre as a form of heart disease, beginning the chapter on "Diseases of the Heart" with the following words: "There is one malady which I have in five cases seen coincident with what appeared to be enlargement of the heart, and which, so far as I know, has not been noticed, in that connection, by medical writers. The malady to which I allude is enlargement of the thyroid gland." Ever since then the importance of cardiac damage in patients with thyrotoxicosis has impressed students of this disease.

Various aspects of the circulation in thyrotoxicosis have been studied to gain more adequate insight into the pathologic physiology of this condition so as to provide a rational basis for treatment. The minute volume output of the heart has been measured by several investigators, but information regarding the velocity of blood flow has not been hitherto available. The purpose of the present investigation was to learn the degree to which the blood flow is accelerated in aspects of the circulation.

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the pulse rate was 76 after subtotal thyroidectomy when the basal metabolic rate was only 13 per cent above the normal.

Venous pressure. No significant deviations from the normal were observed in the venous blood pressures before or after treatment.

Vital capacity of the lungs and respiratory minute volume. The vital capacity of the lungs was diminished in five of the nine patients in the absence of any evidence of circulatory failure. The diminution was an inconstant finding and was not related to the degree of elevation in the basal metabolic rate. With a decrease in the basal metabolic rate toward normal the vital capacity of the lungs tended to increase although this was not apparent in every instance. Before treatment when the basal metabolic rate averaged 33 per cent above normal, the average vital capacity of the lungs was 1870 cc. per square meter of body surface. After compound solution of iodine had been given, the average basal metabolic rate decreased to 22 per cent above normal, but the vital capacity of the lungs failed to increase. After operation, however, the average basal metabolic rate was one per cent above normal and the average vital capacity increased to 2010 cc. per square meter of body surface.

In a few patients the respiratory minute volume was measured while the basal metabolic rate was elevated and again following appropriate treatment. While there was slight diminution in the respiratory minute volume with a return of the basal metabolic rate to normal, the magnitude of the respiratory minute volume before treatment and its decrease after treatment bore no direct relation to the oxygen consumption.

Velocity of blood flow. The velocity of blood flow was strikingly increased, the pulmonary circulation time in some cases being the most rapid observed in any condition up to this time. As in our previous studies, the velocity of blood flow from the arm to the heart showed considerable variation, although in most patients it was definitely increased above the normal. The variability of the arm to heart circulation time was unusually great, due, probably, to the vasomotor instability of these thyrotoxic patients. The extent of the increase in the velocity of blood flow through the lungs was closely related to the extent of increase in the basal metabolic rate. This relationship was present in each individual case and is shown by the average results.

under as nearly basal conditions as possible. The pulse rate was counted several times before and after each test. Whenever feasible, observations were repeated after the basal metabolic rate had returned to normal in order to study the effect of treatment.

RESULTS

Twenty-seven measurements of the pulmonary circulation time and related aspects of the circulation were made in thirteen patients. In studying the data it seemed desirable to divide the patients into two groups. The first group includes nine patients who showed no clinical evidence of circulatory insufficiency; the second group consists of four patients who showed signs of cardiovascular disease.

I. Thyrotoxic patients with no clinical evidence of cardiovascular disease

Table 1 presents the results of twenty measurements of the pulmonary circulation time and related aspects of the circulation in the nine patients of this group, all of whom had exophthalmic goitre. In all but one of the patients, (D. A.), observations were repeated when the basal metabolic rate had been lowered by treatment. The diagnoses were established by the clinical findings and by microscopic examination of the excised thyroid tissue, the results of which are given in the appended case summaries. The clinical condition of the patients varied considerably. Some individuals were very toxic and had experienced symptoms for many years, while in others the disease was less severe and of shorter duration. Six of the nine patients were females, and three patients were males. The ages of the patients varied from 18 to 45 years.

Blood. In all patients the hemoglobin and red blood cell concentration in the peripheral blood were within the limits of normal.

Pulse rate. The pulse rates before treatment were usually elevated but became normal with lowering of the basal metabolic rate. There was a general relation between the degree of elevation of the pulse rate and the increase in the basal metabolic rate. In a given case, however, the relation was not always evident. Patient W. F., for instance, with a basal metabolic rate of 35 per cent above the normal, had a pulse rate of 68 and 80 on two occasions before treatment, while

November 15, 1927.....	M. C.	F.	23	Exophthalmic goitre	94	120	80	2,800	1,590	11.0	17.5	6.5	166	+24	Before treatment. Lugol's solution
March 27, 1928.....	M. C.	F.	23		124	110	75	2,000	1,240	5.0	11.0	6.0	180	+26	M.V. t.i.d. begun April 2, 1928 and
May 29, 1928.....	M. C.	F.	23		76	110	70	2,500	1,600	8.0	23.0	15.0	72	-13	ended April 15, 1928. Subtotal thy-
															roidectomy April 16, 1928. Path-
															ological diagnosis: Hyperplasia of
															thyroid
January 8, 1929.....	I. B.	M.	45	Exophthalmic	92	140	80	4,200	2,700	8.0	14.0	6.0	180	+27	Before treatment. Lugol's solution
May 20, 1929.....	I. B.	M.	45	goitre	68	130	90	4,100	2,400	10.0	21.5	11.5	94	+3	M.X. t.i.d. begun January 11, 1929
															and ended January 21, 1929. Right
															hemithyroidectomy January 22, 1929.
															Left hemithyroidectomy March 18,
															1929. Pathological diagnosis: Hyper-
															plasia of thyroid
March 6, 1928.....	Y. A.	F.	21	Exophthalmic	94	110	70	3,200	2,010	3.0	9.5	6.5	166	+29	Before treatment. Lugol's solution
March 23, 1928.....	Y. A.	F.	21	goitre	82	105	70	2,600	1,630	7.0	15.0	8.0	135	+20	M.X. t.i.d. begun March 22, 1928
April 14, 1928.....	W. F.	M.	38	Thyrototoxicosis	68	110	60	3,600	2,320	8.0	16.5	8.5	127	+35	Before treatment. Lugol's solution
April 24, 1928.....	W. F.	M.	38		80	115	70	3,400	2,260	3.0	10.5	7.5	144	+35	M.X. t.i.d. begun April 15, 1928 and
June 16, 1928.....	W. F.	M.	38		76	90	40	3,500	2,170	8.0	19.0	11.0	98	+13	ended April 26, 1928. Subtotal thy-
															roidectomy April 27, 1928. Path
															ological diagnosis: Hyperplasia of
															thyroid
October 7, 1927.....	F. R.	F.	18	Exophthalmic	88	130	70	1,900	1,370	7.5	16.5	9.0	120	+11	Lugol's solution M.X. t.i.d. begun
April 15, 1928.....	F. R.	F.	18	goitre	80	115	75	2,700	1,940	8.0	19.0	11.0	98	+7	October 5, 1927 and ended October
															14, 1927. Subtotal thyroidectomy
															October 15, 1927. Pathological di-
															agnosis: Hyperplasia of thyroid
Average before treatment.....					99	123	74	2,960	1,870	4.9	10.8	5.9	183	+33	
Average after Lugol's solution.....					83	117	70	2,630	1,753	5.8	14.0	8.2	132	+22	
Average after operation.....					72	112	71	3,220	2,010	8.4	21.5	13.1	83	+1	
Normal average.....					76	120	80	2,250	1,410	6.6	17.4	10.8	100	±0	

TABLE 1

Circulatory measurements and related aspects in patients with thyrotoxicosis who showed no clinical evidence of cardiovascular disease

Date	Name	Sex	Age years	Clinical diagnosis	Pulse rate	Arterial pressure		Vital capacity		Circulation time			Pulmonary circulation velocity, percentage of normal	Basal metabolic rate, percentage variation from normal	Remarks
						Systolic mm. Hg	Diastolic mm. Hg	Observed cc.	Per square meter	Arm to heart sec- onds	Arm to arm sec- onds	Pulmonary sec- onds			
April 13, 1928.....	E. B.	F.	32	Thyrotoxicosis	108	140	80	2,500	1,610	3.5	7.5	4.0	270	+60	Before treatment. Lugol's solution M.X. t.i.d. begun April 15, 1928, and ended April 24, 1928. Subtotal thyroidectomy April 25, 1928. Pathological diagnosis: Hyperplasia of thyroid
June 1, 1928.....	E. B.	F.	32		78	100	70	2,700	1,720	5.5	15.0	9.5	114	+9	
November 15, 1927.....	G. O.	F.	25	Exophthalmic goitre	98	110	70	2,800	1,730	4.5	8.5	4.0	270	+24	Before treatment. Lugol's solution M.X. t.i.d. begun November 15, 1927, and ended November 18, 1927. Subtotal thyroidectomy November 19, 1927. Pathological diagnosis: Hyperplasia of thyroid
March 21, 1928.....	G. O.	F.	25		68	105	70	3,400	2,090	10.0	30.0	20.0	54	-3	
May 17, 1928.....	G. O.	F.	25		66	118	75	3,300	2,000	6.0	18.0	12.0	90	±0	
January 9, 1928.....	D. S.	M.	45	Exophthalmic goitre	105	115	40	3,200	1,960	2.5	8.0	5.5	196	+35	Before treatment. Lugol's solution M.X. t.i.d. begun January 21, 1928, and ended February 3, 1928. Subtotal thyroidectomy February 4, 1928. Pathological diagnosis: Hyperplasia of thyroid
June 16, 1928.....	D. S.	M.	45		60	124	80	3,700	2,200	11.0	29.0	18.0	60	-9	
October 6, 1927.....	D. A.	F.	30	Exophthalmic goitre, myasthenia gravis	120	140	92	1,800	1,190	2.0	8.0	6.0	180	+33	Before treatment

TABLE 2

Circulatory measurements and related aspects in patients with thyrotoxicosis who showed clinical evidence of cardiovascular disease

Date	Name	Sex	Age years	Clinical diagnosis	Pulse rate	Arterial pressure		Vital capacity		Circulation time			Pulmonary circulation velocity, percentage of normal	Basal metabolic rate, percentage variation from normal	Remarks
						Systolic mm. Hg	Diastolic mm. Hg	Observed cc.	Per square meter	Arm to heart sec- onds	Arm to arm sec- onds	Pulmonary sec- onds			
January 8, 1929.....	L. R.	M.	36	Thyrotoxicosis, thyrotoxic heart	90	135	72.3	100	1,970	4.0	10.0	6.0	180	+33	Before treatment
February 13, 1928.....	M. B.	F.	61	Thyrotoxicosis	116	195	110	1,500	960	7.0	13.0	6.0	180	+50	Before treatment. Lugol's solution
March 26, 1928.....	M. B.	F.	61	Hypertension	88	200	110	2,000	1,340	6.5	16.5	10.0	108	+41	M.X. t.i.d. begun on February 13,
May 10, 1928.....	M. B.	F.	61		90	225	120	2,000	1,250	9.0	23.0	14.0	77	+28	1928, and ended on February 20, 1928.
															Subtotal thyroidectomy on February
															21, 1928. Pathological diagnosis:
															Toxic adenoma of thyroid
February 13, 1928.....	R. N.	F.	45	Thyrotoxicosis	94	118	65	1,700	1,080	5.0	12.0	7.0	154	+24	Lugol's solution M.X. t.i.d. begun on
April 17, 1928.....	R. N.	F.	45	Thyrotoxic heart, auricular fibrilla- tion	69	115	70	3,200	1,950	11.0	26.5	15.5	70	+7	February 12, 1928, and ended on
															February 14, 1928. Subtotal thy-
															roidectomy on February 15, 1928.
															Pathological diagnosis: Hyperplasia of
															thyroid
December 6, 1927.....	P. F.	M.	66	? Toxic adenoma, arteriosclerosis, thyrotoxic heart	88	120	80	2,500	1,690	7.5	17.0	9.5	114	+40	Before treatment
Average before operation.....					97			2,200	1,430	5.9	13.0	7.2	150	+37	
Average after operation.....					80			2,600	1,600	10.0	24.8	14.8	73	+18	
Normal average.....					76			2,250		6.6	17.4	10.8	100	±0	

In a previous study of fifty-eight normal persons, the arm to heart circulation time averaged 6.6 seconds, and the crude pulmonary circulation time 10.8 seconds. In these patients with thyrotoxicosis in whom the basal metabolic rate averaged 33 per cent above normal, the arm to heart circulation time averaged 4.9 seconds and the crude pulmonary circulation time 5.9 seconds. These results signify an in-

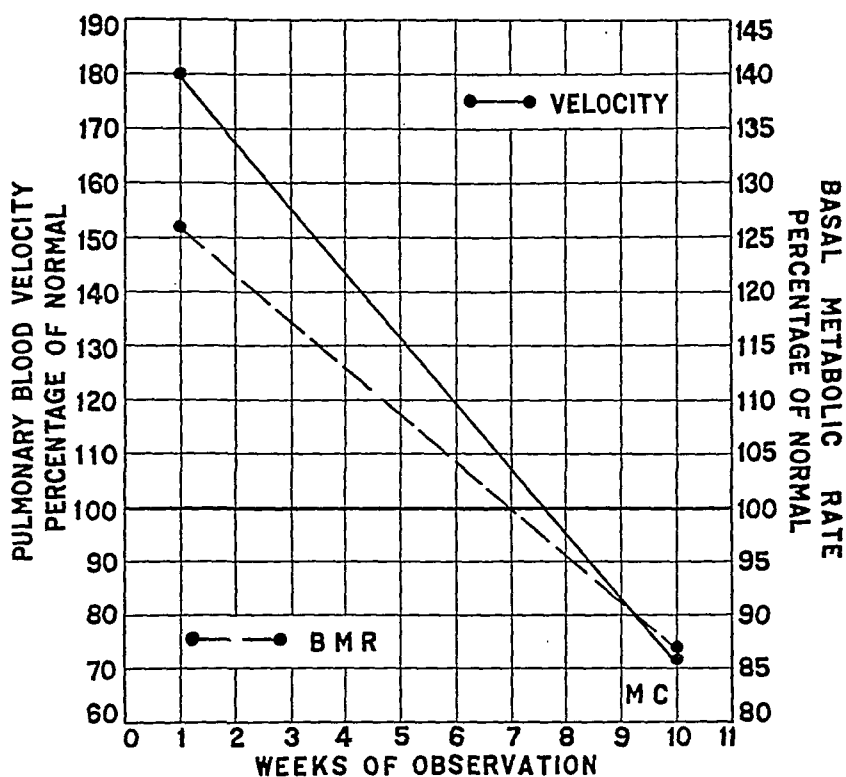


FIG. 1. RELATION OF THE VELOCITY OF BLOOD FLOW THROUGH THE LUNGS AND BASAL METABOLIC RATE IN PATIENT M. C. SUBTOTAL THYROIDECTOMY WAS PERFORMED DURING THE FOURTH WEEK OF OBSERVATION

creased velocity of blood flow from the arm to the heart of 34 per cent and an increased speed of blood flow through the lungs of 83 per cent above the average of normal.

As the basal metabolic rate became lower, the velocity of blood flow likewise approached normal. This is shown graphically in figure 1, as well as by the results in table 1. The slowing in blood flow toward normal as the basal metabolic rate was lowered by Lugol's solution

TABLE 2
Circulatory measurements and related aspects in patients with thyrotoxicosis who showed clinical evidence of cardiovascular disease

Date	Name	Sex	Age years	Clinical diagnosis	Pulse rate	Arterial pressure		Vital capacity		Circulation time			Pulmonary circulation velocity, percentage of normal	Basal metabolic rate, percentage variation from normal	Remarks
						Systolic mm. Hg	Diastolic mm. Hg	Observed cc.	Per square meter	Arm to heart sec- onds	Arm to arm sec- onds	Pulmonary sec- onds			
January 8, 1929	L. R.	M.	36	Thyrotoxicosis, thyrotoxic heart	90	135	72	3,100	1,970	4.0	10.0	6.0	180	+33	Before treatment
February 13, 1928	M. B.	F.	61	Thyrotoxicosis	116	195	110	1,500	960	7.0	13.0	6.0	180	+50	Before treatment. Lugol's solution
March 26, 1928	M. B.	F.	61	Hypertension	88	200	110	2,000	1,340	6.5	16.5	10.0	108	+41	M.X. t.i.d. begun on February 13,
May 10, 1928	M. B.	F.	61		90	225	120	2,000	1,250	9.0	23.0	14.0	77	+28	1928, and ended on February 20, 1928.
															Subtotal thyroidectomy on February
															21, 1928. Pathological diagnosis:
															Toxic adenoma of thyroid
February 13, 1928	R. N.	F.	45	Thyrotoxicosis	94	118	65	1,700	1,080	5.0	12.0	7.0	154	+24	Lugol's solution M.X. t.i.d. begun on
April 17, 1928	R. N.	F.	45	Thyrotoxic heart, auricular fibrilla- tion	69	115	70	3,200	1,950	11.0	26.5	15.5	70	+7	February 12, 1928, and ended on
															February 14, 1928. Subtotal thy-
															roidectomy on February 15, 1928.
															Pathological diagnosis: Hyperplasia of
															thyroid
December 6, 1927	P. F.	M.	66	? Toxic adenoma, arteriosclerosis, thyrotoxic heart	88	120	80	2,500	1,690	7.5	17.0	9.5	114	+40	Before treatment
Average before operation					97			2,200	1,430	5.9	13.0	7.2	150	+37	
Average after operation					80			2,600	1,600	10.0	24.8	14.8	73	+18	
Normal average					76				2,250	6.6	17.4	10.8	100	±0	

affords additional rational basis for the preoperative administration of compound solution of iodine.

II. Patients with thyrotoxicosis and clinical evidences of cardiovascular disease

Seven measurements of the velocity of blood flow and related aspects of the circulation were made in the four patients of this group (table 2). Signs or symptoms of circulatory insufficiency had previously been present but were absent at the time of the test, although in one patient, R. N., auricular fibrillation was present at the time of the first, but not at the time of the second series of measurements. The vital capacity of the lungs was lowered in all patients, while the venous pressure was within the upper limits of normal. The velocity of blood flow through the lungs, although conspicuously increased, was slightly slower than the average velocity observed in the group of patients without cardiovascular disease but with similar basal metabolic rates. The pulse rate was generally increased in proportion to the elevation in the basal metabolic rate as in the preceding group of patients.

DISCUSSION

The work done by the heart consists mainly in expelling the blood into the aorta and into the pulmonary artery against the existing pressures, and in imparting to the blood a certain velocity. The conspicuously increased velocity of blood flow found in patients with thyrotoxicosis emphasizes the strain under which the heart labors even when the body is under basal metabolic conditions.

Certain facts assume increased significance when considered in relation to our results. The hot, flushed, salmon-colored skin, the tendency to perspire, the increased pulse pressure, the tendency to increased blood volume (23) and the diminution in the vital capacity of the lungs observed clinically suggest that considerable vasodilatation is present in thyrotoxicosis and that the functional cross sectional diameter of the peripheral and pulmonary vascular bed is increased. The relation between volume flow and velocity flow through tubes of known diameter is a simple one and is expressed by the equation

$v = \frac{a}{\pi r^2}$ where v = velocity expressed in seconds, a = volume per second and r is the radius of the tube. If other factors remain equal, an increase in the functional cross sectional area of the vascular bed would tend to diminish the speed of blood flow. The fact that the velocity of blood flow is so strikingly increased in spite of the existence of considerable vasodilatation is further evidence of the extreme strain under which the heart labors (24).

Although the second group of patients experienced dyspnoea on the slightest exertion, the velocity of blood flow was only slightly slower than that observed in similar patients (group I) without cardiovascular disease. This fact emphasizes the close interdependence of the circulatory-respiratory-metabolic mechanism. Increased tissue metabolism cannot take place unless there is a proportionate increase both in blood flow and in effective pulmonary ventilation. The observations on the velocity of blood flow in the patients of group I, in whom there was no evidence of cardiovascular disease, indicate the degree to which the velocity of blood flow was increased to satisfy the increased metabolic demands of the tissues. A blood flow less rapid than this in the patients of group II was evidently inadequate and was accompanied by dyspnoea on the slightest exertion. This finding is of interest for while the velocity of blood flow was slightly slower in the patients of group II than in the patients of group I, it was nevertheless much more rapid than that found in normal subjects. The question of whether a given velocity of blood flow is adequate, therefore, cannot be decided in any absolute terms but only in relation to the metabolic rate of that patient. According to this concept the term "normal velocity of blood flow" denotes the velocity of blood flow found in normal subjects with a normal basal metabolic rate.

The extremely rapid velocity of blood flow observed in patients with thyrotoxicosis affords additional information as to why such individuals experience signs and symptoms of circulatory insufficiency on but relatively slight exertion. Plummer and Boothby (25) have shown that a given amount of work by thyrotoxic patients is accompanied by a disproportionate rise in the basal metabolic rate requiring a similar disproportionate rise in ventilation and in blood flow. Rabinowitch (8) and others have shown that the vital capacity of thyrotoxic

patients is greatly diminished, thereby imposing a limitation on the degree to which the ventilation can be increased. The work of Liljestrand and Stenström (13), Bock and Field (26), Kininmonth (15), Burwell, Smith, and Neighbors (17), Means and Newburgh (29) and others indicates that the minute volume output of the heart in thyrotoxic patients at rest corresponds to that in normal individuals doing light work. This indicates that the "reserve" in the minute volume output of the heart is utilized by thyrotoxic patients even while at rest. The extremely rapid blood flow found in the present studies indicates similarly that what may be termed the "reserve" in the velocity of blood flow has been seriously encroached upon. In brief, a thyrotoxic individual experiences dyspnoea more readily than a normal one because: (1) an increased gaseous exchange is necessary; (2) a greater expenditure of energy and hence a relatively greater degree of hyperpnea is necessary to accomplish a given task; (3) the pulmonary bellows are much less efficient; (4) the "reserve" in the minute volume output has been moderately, and the "reserve" in the velocity of blood flow has been greatly encroached upon even while the patient is at rest.

The greatly increased work of the heart even under basal conditions serves to explain the frequency of circulatory insufficiency in thyrotoxicosis. Whether the frequency of cardiac damage in this condition is due partly to a specific toxic effect on the heart cannot be stated on the basis of present knowledge.

The increased velocity of blood flow in thyrotoxicosis probably occurs to meet the demands of the elevated metabolic rate and not as a result of a toxic effect on the heart. We have observed several patients with essential hypertension in whom the basal metabolic rate was elevated as high as plus 33 per cent without the clinical evidence of thyrotoxicosis. Measurements demonstrate that in these subjects the increase in the velocity of blood flow through the lungs is similar to that observed in thyrotoxic patients with equally high metabolic rates but without hypertension. The findings are in accord with certain observations on the minute volume output of the heart (14, 28). The increased burden imposed by this elevation of basal metabolic rate is of serious import to the already overworked heart and indicates the advisability of reducing the basal metabolic rate in such patients

by appropriate means. Such reduction of the basal metabolic rate while tending to lessen the amount of the cardiac work, could not be expected to affect the degree of the arterial hypertension. Similar considerations probably apply to other states such as leukemia and fever in which the metabolic rate is elevated. These observations, again demonstrate that alteration of one fundamental physiological function is accompanied by changes which tend to keep constant the various relationships within the internal environment.

SUMMARY

1. Twenty-seven series of measurements were made in thirteen patients with thyrotoxicosis in order to correlate the clinical manifestations with changes in the velocity of blood flow through the lungs, the basal metabolic rate, pulse rate, venous and arterial pressures and vital capacity of the lungs. Measurements made when the basal metabolic rate was elevated were compared with subsequent measurements when the rate was reduced.

2. There was a general but inexact relation between the degree of elevation of the pulse rate and the increase in the basal metabolic rate.

3. No significant deviations from the normal were observed in the venous blood pressure before or after treatment.

4. Diminution in the vital capacity of the lungs was an inconstant finding. With a decrease in the basal metabolic rate, the vital capacity of the lungs tended to increase.

5. The velocity of blood flow was strikingly increased so that the pulmonary circulation time was the fastest yet recorded in man. The increase in velocity of blood flow through the lungs was proportional to the degree of elevation in the basal metabolic rate. This emphasizes the strain under which the heart labors in thyrotoxicosis.

6. In nine patients with thyrotoxicosis but without circulatory failure, the basal metabolic rate averaged 33 per cent above the normal, while the velocity of blood flow through the lungs averaged 83 per cent above the normal. In four thyrotoxic patients with similar basal metabolic rates but with cardiovascular disease, the velocity of blood flow was slightly slower. The fact that the latter group of patients experienced dyspnoea on slight exertion emphasizes the close interdependence of the circulatory-respiratory-metabolic mechanism.

7. When the basal metabolic rate was lowered by the administration of compound solution of iodine or by operation, the velocity of blood flow was correspondingly slowed.

ABSTRACTS OF HISTORIES AND PHYSICAL EXAMINATIONS OF PATIENTS
WITH THYROTOXICOSIS

E. B. entered the hospital because of nervousness and loss of weight. She had become increasingly nervous during the four years before admission. One year before admission she became distinctly irritable and noted an undue tendency to perspire. Six months before admission a swelling appeared in the front of the neck. During the three weeks preceding her entry to the hospital she had occasional dyspnoea and palpitation. There had been a loss of 17 lbs. in weight during the last six months. *Physical examination* showed marked nervousness; a moist, warm skin; a flushed face; symmetrical enlargement of the thyroid gland with systolic bruit over it; the heart not enlarged, with rate of 100 per minute; fine tremor of fingers and hyperactive reflexes. The blood pressure was 140 mm. Hg systolic and 80 mm. Hg diastolic. The basal metabolic rate was plus 60 per cent on April 12. Ten minims of Lugol's solution were given three times daily from April 15 to April 24. Subtotal thyroidectomy was performed on April 25. The pathological report was "Marked chronic inflammation, with marked follicular hyperplasia, and no colloid distention; hyperplasia of thyroid."

G. O'M. entered the hospital because of nervousness, fatigue and swelling of the neck. For eighteen months before admission she had noticed easy fatigability, restlessness and tremor of the hands. One year before admission she began to perspire unduly and noted a swelling at the base of the neck. She experienced palpitation on slight exertion during the two months before admission. She lost 20 lbs. within the six months preceding her entry to the hospital, although her appetite remained very good. *Physical examination* showed restlessness; quick, jerky movements; warm, moist skin; slight exophthalmos; symmetrical enlargement of the thyroid gland, with a bruit over the isthmus. Her heart was not enlarged, the rate was 94 per minute. The blood pressure was 120 mm. Hg systolic, 70 mm. Hg diastolic. The reflexes were hyperactive and there was a fine tremor of the fingers. The basal metabolic rate was plus 24, plus 22 and plus 24 per cent on October 12, November 10 and November 16, respectively. Ten minims of Lugol's solution were given three times a day from November 15 to November 18. A subtotal thyroidectomy was performed on November 19. The pathological report was—"Sections show marked chronic inflammation, with very marked follicular hyperplasia, the epithelium being columnar in type, and showing retrograde changes. There is no colloid distention; hyperplasia of thyroid."

D. S. came to the hospital because of nervousness, easy fatigability, and trembling of hands, two months in duration. In spite of increased food intake, he had

lost 15 pounds in the two months preceding his entry into the hospital. *Physical examination* showed nervousness; flushed facies; warm, moist skin; slight exophthalmos with definite stare; no thyroid gland enlargement; normal sized heart, with rate of 90 per minute. The blood pressure was 115 mm. Hg systolic and 40 mm. Hg diastolic. There was fine tremor of both hands. The basal metabolic rate was plus 40 and plus 35 per cent on December 28 and January 7, respectively. Ten minims of Lugol's solution were given three times a day from January 21 to February 3. Subtotal thyroidectomy was performed on February 4. The pathological report was "Moderate chronic inflammation with very marked follicular hyperplasia, showing retrograde changes; hyperplasia of thyroid."

D. A. had had a right hemithyroidectomy in 1915, twelve years before the present admission to the hospital. Three years previously drooping of the eyelids was first noted. Four months before admission she had to give up her work on account of increasing weakness and nervousness. Dysphagia and dysarthria gradually developed. Food seemed to lodge in her throat, and her voice became nasal in quality. Two months before admission diplopia was noted, especially on fatigue. She had lost 20 pounds during the four months preceding admission. She reentered now because of nervousness, weakness, difficulty in talking and swallowing, and drooping of the eyelids. *Physical examination* showed complete ptosis of the upper eyelids; limitation of ocular movements laterally and upward; weakness of facial muscles without atrophy; nasal quality to speech; well-healed, semi-circular scar at base of neck; small, hard mass moving with deglutition to the right of the hyoid bone; normal sized heart, with rate of 120 per minute; warm, moist skin and marked tremor of fingers. The blood pressure was 140 mm. Hg systolic and 90 mm. Hg diastolic. The basal metabolic rate was plus 23, plus 32 and plus 31 per cent on September 27, October 10 and 11, 1927, respectively. The clinical diagnosis was not only exophthalmic goitre but also myasthenia gravis. Ten minims of Lugol's solution were given three times daily from October 11 to October 23. Left hemithyroidectomy was performed on October 24. She died October 26. The pathological report was "Moderate chronic inflammation with moderate follicular hyperplasia, the epithelium being columnar in type; hyperplasia of thyroid."

M. C. entered the hospital because of swelling in her neck, palpitation, nervousness and difficult breathing. Four months before admission increased nervousness, voracious appetite and a tendency to perspire freely were noted. Six weeks before admission a swelling in the neck and tremor of the hands appeared. During the month before entry she had experienced palpitation and dyspnoea, especially on lying down. *Physical examination* showed a warm, moist skin; fine tremor of extended hands; moderate exophthalmos with bilateral lid-lag, symmetrical enlargement of thyroid gland with bruit; heart of normal size with rate of 120 per minute and a soft blowing systolic murmur over the apex. The blood pressure

was 136 mm. Hg systolic, 98 mm. Hg diastolic. The basal metabolic rate was plus 28 and plus 24 per cent on November 11 and 16, 1927, respectively. Treatment was delayed because the patient developed an upper respiratory tract infection and an acute purulent otitis media. Ten minims of Lugol's solution were given three times daily from April 2 to April 15, 1928. Subtotal thyroidectomy was done the next day. The pathological report was "Sections show marked chronic inflammation, with marked follicular hyperplasia, the epithelium being columnar in type, and no colloid distention. Parenchymatous hyperplasia of thyroid."

I. B. had suffered from nervousness, loss of weight, tremor of hands, and palpitation for one year before admission to the hospital. During the two months preceding his entry, eight to ten attacks of palpitation occurred daily. He perspired unduly and fatigued easily. *Physical examination* showed nervousness and restlessness. The skin was moist, warm and salmon-colored. There was moderate exophthalmos; an enlarged thyroid gland with a bruit and thrill over it; a normal sized heart with rate of 100 per minute; and marked tremor of fingers. The blood pressure was 148 mm. Hg systolic, 68 mm. Hg diastolic. The basal metabolic rate was plus 44, plus 22 and plus 27 per cent on January 3, 5 and 9, respectively. Ten minims of Lugol's solution were given three times a day from January 11 to January 21. Right hemithyroidectomy was performed on January 22 and left hemithyroidectomy on March 18. The pathological report was "Sections show marked follicular hyperplasia, with marked chronic inflammation. There is no colloid distention of acini. Parenchymatous hyperplasia of thyroid gland."

Y. A. entered the hospital because of increasing nervousness, perspiration and swelling in her neck. A swelling first appeared in the neck seven years before admission. For two years before admission she had been markedly nervous and had suffered from disturbed sleep, and profuse perspiration. *Physical examination* showed a nervous, restless girl with flushed, moist skin; slight exophthalmos with lid-lag; symmetrical enlargement of the thyroid gland with systolic and diastolic bruit over it; normal sized heart with rate of 90 per minute; and a coarse tremor of extended hands. The blood pressure was 120 mm. Hg systolic, 70 mm. Hg diastolic. The basal metabolic rate was plus 31 and plus 29 per cent on March 1 and 5, respectively. Ten minims of Lugol's solution were given three times daily from March 22 to April 3. Subtotal thyroidectomy was performed April 4. The pathological report was "Marked chronic inflammation, with marked follicular hyperplasia, but no colloid distention. Hyperplasia of thyroid."

W. F. entered the hospital because of nervousness and loss of weight. For one year before admission he had tired easily, had become irritable and nervous, and had developed a tremor of the hands. In spite of greatly increased food intake

he had lost 20 pounds in the three months before admission. *Physical examination* revealed a flushed, moist skin; marked tremor of extended hands; heart not enlarged with rate of 120 per minute. The blood pressure was 120 mm. Hg systolic, 60 mm. Hg diastolic. There was no exophthalmos or enlargement of the thyroid gland. The basal metabolic rate was plus 36 and plus 34 per cent on April 12 and 16, respectively. Ten minims of Lugol's solution were given three times daily from April 15 to April 26. Subtotal thyroidectomy was performed on April 27. The pathological report was "Sections show moderate follicular hyperplasia, with slight chronic inflammation. There was no colloid distention. Hyperplasia of thyroid (moderate)."

F. R. entered the hospital because of tremor of hands and nervousness. Two years before admission undue irritability and nervousness developed and one year before admission she noted tremor of hands and voracious appetite. *Physical examination* showed trembling of lips and chin; a warm, moist skin with pigmented areas under chin and in right axilla; slight exophthalmos; a palpable thyroid gland; normal heart with rate of 90 per minute; and fine and coarse tremor of both hands. The blood pressure was 130 mm. Hg systolic, 70 mm. Hg diastolic. The basal metabolic rate was plus 35 per cent on September 20 and plus 10 per cent on October 7. Thirty minims of Lugol's solution were given daily from October 5 to October 14. Subtotal thyroidectomy was performed on October 15, 1927. The pathological report was "Marked chronic inflammation, with moderate follicular hyperplasia. Epithelium columnar in type, and there are retrograde changes in acini. Hyperplasia of thyroid."

L. R. entered the hospital because of attacks of palpitation, dyspnoea and loss of weight. One year before admission attacks of palpitation gradually increasing in severity appeared. About five months before admission he began to suffer from dyspnoea on exertion with occasional attacks of severe palpitation. *Physical examination* showed a young man with a flushed, moist, salmon-colored skin, and tremor of hands. There was no exophthalmos. The thyroid gland was uniformly enlarged. The heart showed moderate enlargement to the left, action regular, rate 85 per minute. The first sound was accentuated and a systolic murmur was heard over the apex and base. The blood pressure was 135 mm. Hg systolic and 65 mm. Hg diastolic. Electrocardiographic tracings on admission showed normal rhythm, a week later, auricular fibrillation. The basal metabolic rate was plus 68 and plus 33 per cent on December 14, 1928 and January 8, 1929, respectively. Thirty minims of Lugol's solution were given daily from January 10 to January 21. Right hemithyroidectomy was performed on January 22 and and left hemithyroidectomy on March 26, 1929. The pathological diagnosis, was "Parenchymatous hyperplasia of thyroid gland."

M. B. had suffered from nervousness, dizziness and trembling of hands for one year and marked weakness for six months. During these six months she perspired

profusely and lost 25 pounds. *Physical examination* showed flushed skin and face, moderate exophthalmos with lid-lag, symmetrical enlargement of thyroid gland and fine tremor of extended hands. The heart was moderately enlarged to the left, the rate, 110 per minute. No murmurs were heard. The peripheral vessels were sclerosed and the blood pressure was 210 mm. Hg systolic and 110 mm. Hg diastolic. There were no signs of congestive heart failure. Electrocardiographic tracings showed left ventricular predominance with slurring of R₁ and S₃. The basal metabolic rate was plus 50 per cent on February 14. Lugol's solution, ten minims, three times a day, was given from February 13 to February 21. A subtotal thyroidectomy was performed February 21. The pathological report was "Sections show marked colloid distention of acini, with slight chronic inflammation. There is no evidence of hyperplasia. Toxic adenoma of thyroid gland." On March 26 she complained of weakness and trembling of hands, the blood pressure was 200 mm. Hg systolic and 110 mm. Hg diastolic, and there was slight pitting edema of lower extremities. The lungs were clear. The basal metabolic rate was plus 41 per cent. On May 10 she felt much better, had gained 22 pounds and was no longer nervous. The blood pressure was 225 mm. Hg systolic and 120 mm. Hg diastolic. The basal metabolic rate was plus 28 per cent.

R. N. entered the hospital because of attacks of palpitation, nervousness and easy fatigability, one year in duration. She had lost about 60 pounds during the year before admission, in spite of increased food intake. *Physical examination* showed a prematurely gray-haired woman; with flushed, moist skin; moderately enlarged thyroid gland; normal sized heart with rate of 94 per minute and absolutely irregular rhythm; a pulse deficit of 12; and fine tremor of extended hands. There was no exophthalmos, lid-lag, or peripheral edema. Electrocardiogram on February 13 showed auricular fibrillation. The basal metabolic rate was plus 22 and plus 24 per cent on February 10 and 14, respectively. Lugol's solution, ten minims, three times daily, was given from February 12 to February 14. Subtotal thyroidectomy was performed February 15. The pathological report was "Moderate to marked chronic inflammation. Follicular hyperplasia is very marked and the epithelium shows retrograde changes. There is no colloid distention. Hyperplasia of thyroid."

P. F. entered the hospital because of cough, dyspnoea and edema of feet. For many years he had suffered from "asthma." Three years before admission increasing nervousness developed. One month before admission sensation of pressure over the precordium, cough, and nocturnal dyspnoea appeared. Three days before admission he noticed swelling of his feet and palpitation on the slightest exertion. In spite of increased food intake, he had lost weight steadily. *Physical examination* showed a poorly nourished, elderly white man, restless and apprehensive; with marked exophthalmos; a warm, moist skin;

palpable thyroid gland with a hard nodule in the right lobe. The heart was definitely enlarged with systolic murmur over apex, rate of 90 per minute, and absolutely irregular rhythm. Peripheral vessels were markedly sclerosed. Auscultation of lungs showed prolonged expiratory phase accompanied by musical squeaks. There was pitting edema of both legs up to the knees. The blood pressure was 120 mm. Hg systolic and 80 mm. Hg diastolic. With full doses of digitalis, the edema disappeared and normal sinus rhythm was restored by the administration of quinidine sulphate. The basal metabolic rate was plus 29 and plus 40 per cent on November 5 and December 3, respectively. Ten minims of Lugol's solution were given three times daily from December 14 to December 22, 1927. Right hemithyroidectomy was performed December 23, 1927 and left hemithyroidectomy on February 9, 1928. The pathological report was "Sections show moderate chronic inflammation but marked follicular hyperplasia with columnar epithelium, showing retrograde changes. No colloid distention seen. Hyperplasia of thyroid."

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STUDIES ON THE VELOCITY OF BLOOD FLOW

XIV. THE CIRCULATION IN MYXEDEMA WITH A COMPARISON OF THE VELOCITY OF BLOOD FLOW IN MYXEDEMA AND THYROTOXICOSIS¹

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The clinical aspects of the circulation in myxedema have aroused widespread interest, but knowledge of the pathologic physiology of the blood flow in this condition is meagre. The present study undertakes the correlation of the clinical manifestations of myxedema with changes in the velocity of blood flow, basal metabolic rate, pulse rate, plasma volume, venous and arterial pressures, respiratory minute volume and vital capacity of the lungs. The same methods were employed as in the preceding study of thyrotoxicosis (1).

RESULTS

Sixteen series of measurements were made in seven consecutive patients with the classical clinical manifestations of spontaneously occurring myxedema (table 1). In each patient measurements made when the basal metabolic rate was low were compared with subsequent measurements when the basal metabolic rate had been elevated to normal by appropriate doses of desiccated thyroid gland by mouth. The clinical findings in the patients are summarized in the appended abstracts. All subjects were women between the ages of 45 and 58 years, except subject E. Sa. who was 18 years old. The tendency of myxedema to occur predominantly in women is well recognized.

Blood. "Secondary" anemia was observed on examination of the

¹ This investigation was aided in part by a grant from the DeLamar Mobile Research Fund of Harvard University.

TABLE 1

Circulatory measurements and related aspects in patients with myxedema

Date	Name	Sex	Age years	Hemoglobin per cent	Red blood cells mil- lions per cu. mm.	Plasma volume per kilo	Pulse rate	Arterial pressure		Vital capacity		Respiratory volume per square meter	Circulation time			Pulmonary circulation percentage of normal	Basal metabolic rate, percentage variation from normal	Treatment
								Systolic mm. Hg	Diastolic mm. Hg	Observed cc.	Per square meter		Arm to heart sec- onds	Arm to arm sec- onds	Pulmonary sec- onds			
December 5, 1927.....	E. St.	F.	50	66	64	100	60	2,800	1,660	12.5	38.5	26.0	42	-22	Received a total of 11.8 grams desic- cated thyroid from December 6, 1927 to December 28, 1927			
December 28, 1927.....	E. St.	F.	50	67	88	100	60	2,600	1,560	10.0	20.0	10.0	108	+1				
January 18, 1928.....	E. St.	F.	50	72	63	100	65	2,650	1,600	14.5	28.0	13.5	80	-13	Received a total of 11.6 grams desic- cated thyroid from March 15, 1928 to March 26, 1928			
March 14, 1928.....	M. K.	F.	58	70	69	130	90	2,450	1,600	4.4	5.0	29.0	45	+6				
March 26, 1928.....	M. K.	F.	58	60	120	115	85	1,800	1,230	5.6	4.0	11.0	7.0	154				
February 22, 1928.....	M. G.	F.	53	73	60	140	90	2,600	1,580	2.9	10.0	30.0	20.0	54	-25	Received a total of 12.0 grams desic- cated thyroid from February 24, 1928 to March 6, 1928		
March 6, 1928.....	M. G.	F.	53	87	84	135	90	2,500	1,550	3.8	11.0	21.5	10.5	103	-3			
October 4, 1928.....	R. F.	F.	54	74	56	120	70	2,100	1,220	2.6	8.0	25.0	17.0	64	-24	Received a total of 12.8 grams desic- cated thyroid from October 4, 1928 to October 26, 1928		
October 26, 1928.....	R. F.	F.	54	79	100	130	85	1,850	1,100	3.6	11.0	17.5	6.5	166	+14			
February 20, 1929.....	S. M.	F.	48	75	62	125	80	2,850	1,590	2.7	12.0	27.0	15.0	72	-21	Received a total of 11.3 grams desic- cated thyroid from February 21, 1929 to March 14, 1929		
March 15, 1929.....	S. M.	F.	48	74	82	130	70	2,800	1,560	3.1	9.0	18.0	9.0	120	-1			
March 27, 1928.....	M. M.	F.	45	80	66	120	78	2,200	1,300	2.2	7.5	22.5	15.0	72	-33	Received a total of 16.4 grams desic- cated thyroid from March 28, 1928 to April 16, 1928		
April 16, 1928.....	M. M.	F.	45	86	90	120	80	2,500	1,530	3.8	6.0	12.0	6.0	180	±0			
April 24, 1928.....	M. M.	F.	45	73	112	125	80	2,300	1,420	3.7	5.0	13.0	8.0	135	+12	Received a total of 20.0 grams of desic- cated thyroid from March 6, 1928 to March 26, 1928		
March 6, 1928.....	E. Sa.	F.	18	88	76	100	70	3,000	1,660	4.3	10.0	23.0	13.0	83	-23			
March 26, 1928.....	E. Sa.	F.	18	88	87	110	70	2,700	1,540	4.5	3.5	11.5	8.0	135	-2			
Average before treatment.....				75	4.0	34.1	65	119	77	2,570	1,510	3.2	9.6	27.9	18.6	58	-24	
Average after treatment.....				77	4.0	37.6	93	120	77	2,390	1,440	4.1	7.8	15.9	8.1	133	+2	
Average normal.....				100	4.5	50.0	76	120	80	2,000		3.7	6.6	17.4	10.8	100	±0	

blood of every patient. No significant change in the degree of anemia was noted in the relatively short time that intervened between the blood flow measurements before and after thyroid treatment. The occurrence of anemia in myxedematous patients and its tendency to disappear with thyroid therapy have been noted by Emery (2) and others. Our patients were not studied over a sufficient length of time to observe a return of the blood to normal. The dilution of the blood with the increase in plasma volume which occurred following thyroid medication evidently obscured any slight increase in the absolute number of red blood cells. This is particularly evident in patient M. K. (table 1).

The plasma volume was measured both before and after treatment with thyroid extract by the brilliant vital red method used by Thompson (3). Our findings agree in general with those of Thompson who found that the plasma volume per kilogram of body weight is low in myxedema and that a significant increase occurs on administration of thyroid gland. The average plasma volume per kilo of the 7 cases before treatment was 34.1 cc. compared with 37.6 cc. after treatment. A parallelism between the changes in basal metabolic rate and plasma volume was not observed always; indeed the plasma volume per kilo decreased or remained unchanged in two of seven patients after administration of thyroid gland.

Pulse rate and pulse pressure. The pulse rate was low in the patients with myxedema and bore a general relation to the basal metabolic rate although an exact parallelism was not present in each case. The pulse rate averaged 65 per minute before treatment compared with an average of 93 following adequate thyroid extract therapy. Likewise, the pulse pressure was small when the basal metabolic rate was low and tended to increase somewhat after treatment. These observations are similar to those of Davies and Eason (4) and to those of Willius and Haines (5) who noted an average increase in the pulse pressure from 36 mm. mercury before treatment to 45 mm. after treatment.

Venous pressure. The venous pressure was within the limits of normal in all our patients, a fact which is in accord with the absence of clinical manifestations of congestive failure.

Vital capacity of the lungs. In all subjects, the vital capacity of the

lungs was strikingly diminished in the absence of any signs of congestive failure and did not show a significant change following treatment. We are unable to explain this phenomenon. The extent of diminution in the vital capacity was not closely related to the degree of lowering in the basal metabolic rate.

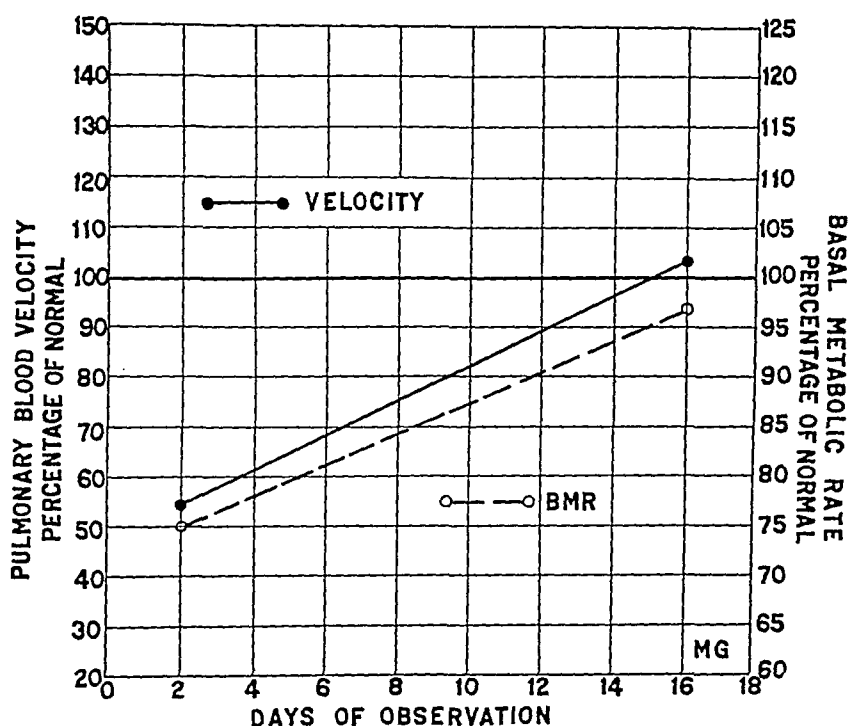


FIG. 1. THE RELATION BETWEEN PERCENTAGE VARIATIONS IN THE BASAL METABOLIC RATE AND THE VELOCITY OF BLOOD FLOW THROUGH THE LUNGS IN PATIENT M. G. BEFORE TREATMENT AND AFTER TREATMENT

The circles (o) denote observations of the basal metabolic rate, the solid dots (●), observations on the pulmonary velocity of blood flow.

Respiratory minute volume. The respiratory minute volume was conspicuously decreased before treatment and always rose significantly as the basal metabolic rate increased. The average respiratory minute volume per square meter of body surface before treatment was 3.2 liters compared with 4.1 liters after treatment.

The velocity of blood flow. The velocity of blood flow was strikingly

slow in each of the seven cases of myxedema. This was evident in both the arm to heart blood flow (the arm to heart circulation time) and the blood flow through the lungs (the pulmonary circulation time). As has been observed in all preceding studies (6, 7, 8), the arm to heart circulation time shows considerable variation because of the relatively great spontaneous fluctuations in the arm blood flow. The degree of slowing in the blood flow through the lungs corresponded closely with the degree to which the metabolic rate was lowered. After taking adequate amounts of desiccated thyroid gland by mouth, the rise in the metabolic rate and the increase in the velocity of blood flow to normal took place simultaneously and closely paralleled each other.

These findings are illustrated by the results in patient, M. G. (fig. 1). Before treatment, the basal metabolic rate was 25 per cent below the average normal, and the pulmonary circulation time was .20 seconds, denoting a slowing of the blood flow to but 54 per cent of the average normal speed. The close correspondence between changes in the velocity of blood flow and basal metabolic rate is shown by the measurements after 14 days of treatment, when the basal metabolic rate and velocity of blood flow had both returned to within 3 per cent of the average normal values.

A similar relation was observed in the measurements in patient E. S. (fig. 2). The first measurements were made before treatment when the basal metabolic rate was 22 per cent below the average normal, while the second series of tests was made after the patient had received dried thyroid gland and the basal metabolic rate had risen to 1 per cent above the average normal. Treatment was then discontinued and a third series of observations was made when the basal metabolic rate was minus 15 per cent.

The slowing of the blood flow in myxedema was not entirely unexpected but the degree of slowing was striking, being almost as great as that observed in patients with rheumatic valvular heart disease and auricular fibrillation, who had previously suffered from severe circulatory decompensation and showed symptoms or signs of congestive failure at the time of the test (7). The fact that the myxedematous patients showed no evidence of circulatory insufficiency with a speed of blood flow approximately the same as that of the latter group again emphasizes the fact that the question of whether a given speed of blood

flow is adequate can be decided only in relation to the metabolic needs of the tissues (1).

None of the myxedematous patients showed evidence of cardiovascular disease. Zondek (9), Assmann (10), Fahr (11) and others have described a form of heart failure characteristic of myxedema which

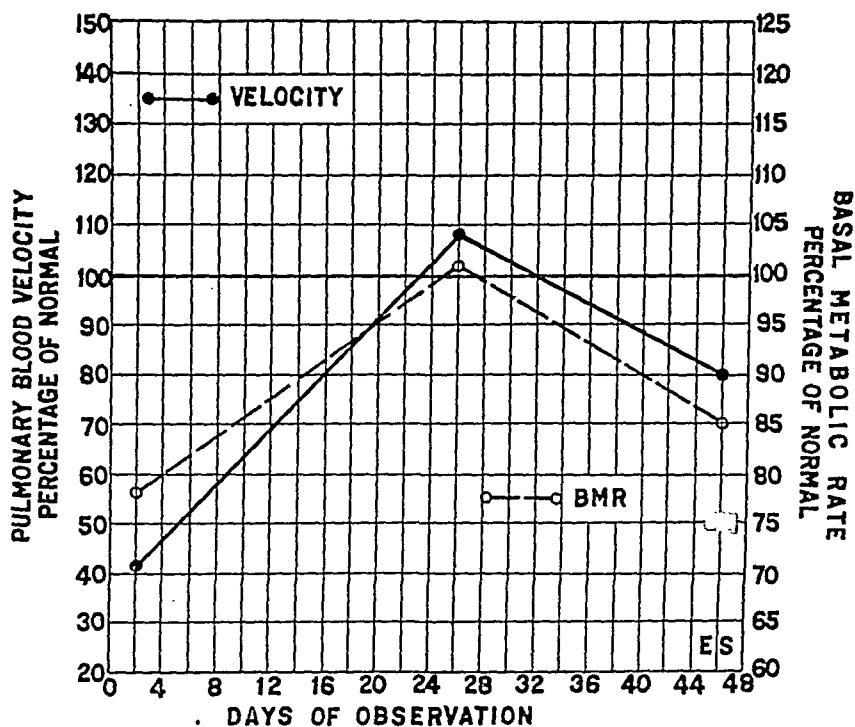


FIG. 2. THE RELATION BETWEEN PERCENTAGE VARIATIONS IN THE BASAL METABOLIC RATE AND THE VELOCITY OF BLOOD FLOW THROUGH THE LUNGS IN PATIENT E.S.

Desiccated thyroid gland was given during the first 24 days of observation. The third pair of observations was made after thyroid medication had been discontinued. The circles denote observations of the basal metabolic rate, the solid dots, observations of the velocity of blood flow through the lungs.

is alleviated only by thyroid therapy. On the other hand, Means, White and Krantz (12) in a study of 48 patients with myxedema encountered but one such case. Willius and Haines (5) in 162 cases found no evidence of heart failure or organic cardiovascular disease that

could be attributed to the presence of myxedema, and Christian (13) similarly states he has never observed the condition.

More numerous examples of the opposite course of events are available, namely, thyroid therapy precipitating circulatory insufficiency rather than alleviating it. Swan (14) has reported the case of a patient with myxedema in whom fibrillation of the auricles appeared whenever thyroid substance was administered, the heart action returning to normal whenever the drug was discontinued. Read (15), and Means, White and Krantz (12) have reported cases in which the administration of thyroid substance caused attacks of angina pectoris; and Sturgis and Whiting (16), and Pratt and Morton (17) observed cases in which at each attempt to give thyroid gland, the signs of congestive failure appeared. Christian (18) has laid particular emphasis on the danger of increasing the heart action of certain myxedematous patients by thyroid gland medication.

The results of our studies offer a rational explanation for these clinical manifestations of cardiac insufficiency following thyroid gland therapy. The great increase in blood velocity that occurs when the metabolic rate is raised to normal necessitates a conspicuously increased amount of work by the heart. Because of this and because the metabolic needs of the myocardium have risen along with those of the rest of the body, the blood supply to the heart must be increased. With the frequent occurrence of hypertension and arteriosclerotic narrowing of the coronary vessels noted by Fishberg (19) and others in cases of myxedema, the necessity for cautious administration of thyroid substance and the frequent advisability of previous digitalization is apparent.

A comparison of changes in the pulse rate, in the basal metabolic rate, and in velocity of blood flow in myxedema and thyrotoxicosis. In this and the preceding communication (1), attention has been directed to the relation between the clinical findings and the measurements of the velocity of blood flow and related aspects of circulation. In the following more general treatment of the data, the relation between changes in the velocity of blood flow, in the pulse rate and in the basal metabolic rate in myxedema and thyrotoxicosis will be compared.

In figures 3, 4 and 5 each dot represents an observation in a patient either before or after treatment. The results that approach normal

values represent findings in thyrotoxic patients after thyroidectomy or in myxedematous patients after thyroid substance had been administered.

The relation between the basal metabolic rate and the pulse rate. Figure 3 shows the relation between the pulse rate and the basal metabolic

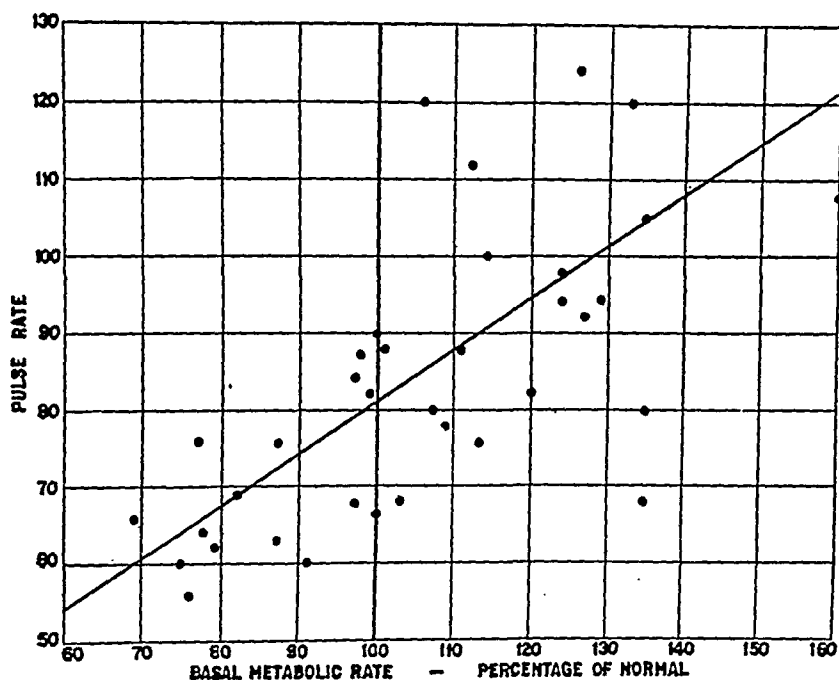


FIG. 3. THE RELATION BETWEEN THE PULSE RATE AND THE BASAL METABOLIC RATE IN PATIENTS WITH MYXEDEMA AND THYROTOXICOSIS

The observations that approach normal in this and the following figures represent findings in thyrotoxic patients after thyroidectomy or in myxedematous patients after thyroid substance had been administered.

rate in patients with myxedema or thyrotoxicosis both before and after treatment. While a general correlation is apparent, the variations of individual measurements are considerable. A similar relationship between the basal metabolic rate and the pulse rate has been noted by Sturgis and Tompkins (20) in patients with thyrotoxicosis and by Minot and Means (21) in patients with chronic leukemia. The observed variations are probably due to the fact that in order to increase

the minute volume output of the heart to meet increased metabolic demands both the stroke volume and the number of beats per minute are increased but in varying proportion in different patients. Similar variations in response can be observed in normal persons performing a standard exercise test (22).

The relation between the pulse rate and the velocity of blood flow through the lungs. The relation between the velocity of blood flow through the lungs and the pulse rate (fig. 4) shows considerable variation, but is

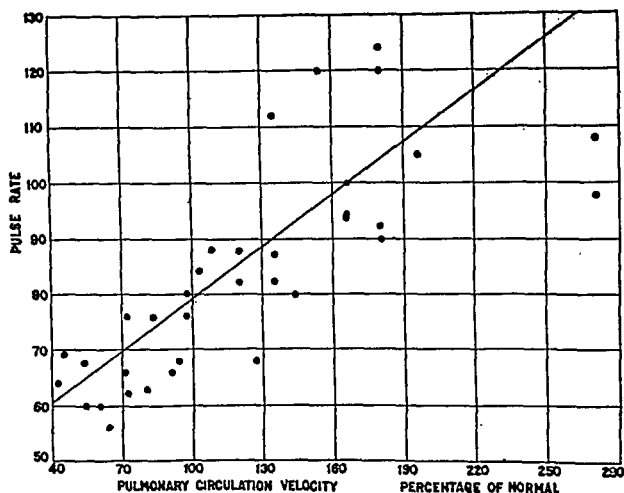


FIG. 4. THE RELATION BETWEEN THE PULSE RATE AND PERCENTAGE VARIATIONS IN THE VELOCITY OF BLOOD FLOW THROUGH THE LUNGS

closer than that between the basal metabolic rate and the pulse rate. This is in accord with what occurs in normal subjects (6). Of 58 normal subjects studied, the ventricular rate was above 90 per minute in 10, while it was below 70 in 8. The ventricular rate of the former group averaged 97 per minute and the pulmonary blood velocity averaged 29 per cent above the average normal, while the ventricular rate of the latter group averaged 65 per minute and the pulmonary blood velocity was 4 per cent below the average normal.

The relation between the basal metabolic rate and the velocity of blood flow through the lungs. The relation between the basal metabolic rate and the velocity of blood flow through the lungs is close and shows relatively few variations (fig. 5). This close parallelism between velocity of blood flow and metabolism is further evidence that the velocity of blood flow is a fundamental characteristic of the circulation. Comparison of the relation between the velocity of blood flow and the

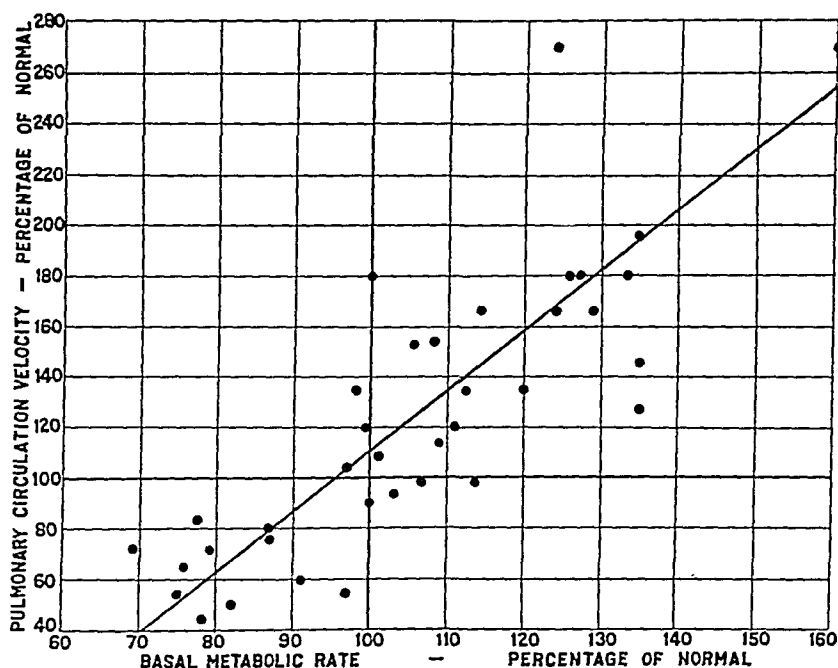


FIG. 5. THE RELATION BETWEEN PERCENTAGE VARIATIONS IN THE PULMONARY BLOOD VELOCITY AND BASAL METABOLIC RATE IN PATIENTS WITH MYXEDEMA AND THYROTOXICOSIS

basal metabolic rate in myxedema and thyrotoxicosis affords additional evidence that the increased velocity of blood flow through the lungs in thyrotoxicosis is due to the elevated metabolism rather than to a specific toxic effect on the heart. The relation between the basal metabolic rate and the velocity of blood flow is a simple linear one. (See fig. 5.) If the mean increase in the velocity of blood flow in thyrotoxicosis were due to a specific toxic effect, the line representing

the relation between the velocity of blood flow and basal metabolic rate would assume a different direction from that for myxedema. The fact that the slope is a continuous one is indirect evidence that the increased blood velocity in thyrotoxicosis is due to the increased basal metabolic rate.

It should be noted that a given percentage increase or decrease in the basal metabolic rate is accompanied by a far greater percentage change in the velocity of blood flow. This finding is in accord with observations on the relation between the minute volume output of the heart and the basal metabolism in myxedema and thyrotoxicosis (23, 24, 25, 26). This does not necessarily mean that there is a disproportionately increased velocity of blood flow in thyrotoxicosis, or a disproportionate decrease in myxedema, for the comparison involves two different phenomena which are expressed in totally dissimilar units. The results demonstrate, however, the close interrelation between the two fundamental physiological characteristics, blood flow and metabolism, and throw additional light on the degree, manner, and results of changes in the circulation associated with increased and decreased metabolic rates.

SUMMARY

1. Sixteen series of measurements were made in 7 patients with myxedema in order to correlate the clinical manifestations with changes in the velocity of blood flow, basal metabolic rate, pulse rate, plasma volume, venous and arterial pressures, respiratory minute volume and vital capacity of the lungs.

2. In each patient measurements when the basal metabolic rate was low were compared with subsequent measurements when the basal metabolic rate had been elevated to normal by appropriate doses of thyroid gland by mouth.

3. The plasma volume per kilogram of body weight was low and tended to increase on administration of thyroid gland.

4. The pulse rate was low and bore a rough relationship to the basal metabolic rate. As the metabolism rose the pulse rate approached normal.

5. The venous pressure was within the limits of normal in all 7 patients.

6. The vital capacity of the lungs was strikingly diminished in all subjects in the absence of any signs of congestive heart failure and did not show significant change following treatment. The extent of diminution in the vital capacity was not closely related to the degree of lowering in the basal metabolic rate.

7. The respiratory minute volume was decreased before treatment and always rose significantly as the basal metabolic rate increased.

8. The velocity of blood flow was strikingly slow in every subject and corresponded closely with the degree to which the metabolic rate was lowered. After taking thyroid gland by mouth, the rise in the metabolic rate and the increase in the velocity of blood flow to normal took place simultaneously and closely paralleled each other.

9. The slowing of blood flow in myxedema was almost as great as that observed in patients with rheumatic valvular heart disease with auricular fibrillation and symptoms and signs of congestive failure. None of the myxedematous patients showed clinical evidences of cardiovascular disease.

10. The great increase in velocity of blood flow and consequent increased cardiac work that occurs when the basal metabolic rate is raised to normal affords a rational explanation of the clinical manifestations of cardiac insufficiency which occur not infrequently following thyroid gland therapy in myxedema.

11. The changes in the pulse rate, basal metabolic rate and velocity of blood flow in myxedema are compared to those previously reported in thyrotoxicosis. The comparison indicates that the increased velocity of blood flow in thyrotoxicosis is due to the increased basal metabolic rate rather than to a specific toxic effect on the heart.

12. The findings emphasize the close interrelation between blood flow and metabolism and throw additional light on the degree, manner, and results of changes in the circulation associated with increased and decreased metabolic rates.

ABSTRACTS OF HISTORIES AND PHYSICAL EXAMINATIONS OF PATIENTS WITH MYXEDEMA

E. St. entered the hospital because of weakness. For two years before admission she noted that she thought and acted in a much more retarded manner than formerly. She had gained considerable weight, and her hair had become dry and brittle. One

year before admission, hearing became impaired and the voice became husky. She began to be very sensitive to cold, and required much clothing even in the warmest weather. On *physical examination* the complexion was pasty, speech thick and slow, the hair sparse and dry, the skin dry, scaly, thickened, the conjunctivae and mucous membranes pale, and the tongue thick and large. The heart was not enlarged. The ventricular rate was 60 per minute, the blood pressure 100 mm. Hg systolic and 60 mm. Hg diastolic. The basal metabolic rate was minus 24 per cent and minus 22 per cent on the 3rd and 6th of December, respectively. Thyroid gland medication was given from December 6 to December 28, 1927. During this period she received a total of 11.8 grams of desiccated thyroid gland. The basal metabolic rate on December 29 was plus 1 per cent.

M. K. had suffered from increasing weakness and easy fatigability for one year. Ten months before admission to the hospital she noticed puffiness of her face, and dryness and roughness of the skin. She also became increasingly sensitive to cold weather. For three months she had noticed that she spoke slowly and that her memory had become distinctly impaired. *Physical examination* showed a puffy and pasty face; gray, coarse, dry hair; dry scaly skin; thickened lips; moderately advanced generalized arteriosclerosis; no cardiac enlargement, a ventricular rate of 70 per minute. She talked very slowly. The blood pressure was 130 mm. Hg systolic and 90 mm. Hg diastolic. The basal metabolic rate was minus 18 per cent on March 13. A total of 11.6 grams of desiccated thyroid gland was given from March 15 to March 26. The basal metabolic rate on the latter date was plus 6 per cent.

M. G. had been treated for myxedema for about five years but six months before our observations she stopped taking thyroid extract. Soon thereafter she developed weakness, puffiness of her face and extreme sensitivity to cold. *Physical examination* showed a well nourished, middle aged woman, with slow deliberate speech; dry, sparse hair; dry, thickened, pasty skin; puffiness under eyes; normal sized heart, with a rate of 60 per minute. The blood pressure was 130 mm. Hg systolic and 80 mm. Hg diastolic. The basal metabolic rate was minus 25 per cent on February 20. Thyroid gland medication was given from February 24 to March 6. During this period she received a total of 12.0 grams of desiccated thyroid gland. The basal metabolic rate on March 6 was minus 3 per cent.

R. F. entered the hospital because of weakness, dizziness and headaches. She had suffered from weakness for one year before admission. About ten months before admission she became very sensitive to cold. Two months before admission she noted increasing dryness of the skin and puffiness of the face. During the week preceding entry to the hospital she had experienced almost constant headache and dizziness. *Physical examination* showed an obese, middle aged woman, with dry coarse hair, puffy face, slow halting speech, dry thick skin, large dry tongue and pads of fat over the clavicles, shoulder blades and hips. The heart was not

enlarged. The ventricular rate was 58 per minute and the blood pressure was 95 mm. Hg systolic and 65 mm. Hg diastolic. The basal metabolic rate was minus 24 per cent. Thyroid gland medication was given from October 4 to October 26. Twelve and eight tenths grams of desiccated thyroid gland were given. The basal metabolic rate on October 27 was plus 14 per cent.

S. M. had suffered from fatigue, lack of ambition, irritability and numbness of fingers and toes for five months. During the four months preceding her entry into the hospital she gained weight steadily without eating more than usual. Her skin had become thick and dry, and she became very sensitive to cold. Two months before admission her hair began to fall out and her irritability of temperament increased, so that she found it hard to get along with people. On *physical examination* she showed a dry, thickened, sallow skin; gray, scanty, dry hair; a heart rate of 60 per minute, and a blood pressure of 120 mm. Hg systolic and 70 mm. Hg diastolic. The basal metabolic rates were minus 24 per cent and minus 21 per cent on February 18 and 21, respectively. Thyroid gland medication was begun on February 21 and ended on March 14. She received a total of 11.3 grams of desiccated thyroid gland. On March 15, the basal metabolic rate was minus 1 per cent.

M. M. had been treated for myxedema for one year. Thyroid gland taken by mouth relieved all symptoms but on cessation of medication five months before entry she gained weight, and became very sensitive to cold. She noted puffiness of the face and tired easily. *Physical examination* showed an obese, middle aged woman with pasty complexion, puffy face, slow mentality and slow speech. Her hair was dry, coarse, and very scanty. The skin was thickened and had a peculiar waxy appearance. The heart was of normal size, with a ventricular rate of 70 per minute. The blood pressure was 115 mm. Hg systolic and 70 mm. Hg diastolic. The basal metabolic rate was minus 33 per cent on March 27. Thyroid gland medication was given from March 28 to April 16. She received a total of 16.4 grams of desiccated thyroid gland during this period. The basal metabolic rate was exactly the average of normal on April 16.

E. Sa. entered the hospital because of excessive gain in weight, constipation, anorexia, and dry hair and skin. She had been feeling well until three months before admission when she began to suffer from obstinate constipation and loss of appetite. She continued to gain weight, however. Then she noted that her hair and skin were getting dry, and that her hair was falling out rapidly. *Physical examination* showed obesity, with fat pads over her clavicles, scapulae and hips; a thickened dry skin, and coarse, sparse, dry hair; a normally sized heart with ventricular rate of 70 per minute and blood pressure of 100 mm. Hg systolic and 70 mm. Hg diastolic. The basal metabolic rate was minus 25 per cent and minus 23 per cent on February 29 and March 2, respectively. Thyroid medication was started on March 6, and omitted on March 26. She received a total of 20.0 grams of desiccated thyroid during this period. The basal metabolic rate on March 26 was minus 2 per cent.

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THE DIAGNOSTIC VALUE OF DETERMINATIONS OF PEPSIN IN GASTRIC JUICE

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In previous papers (1) we described a method for the measurement of pepsin in gastric juice and reported the results of determinations made at frequent intervals before and after stimulation of the stomach secretions by histamine. It was shown that the concentration of pepsin falls after stimulation although the total output of ferment is increased at the height of secretion. It was also pointed out that the curve of pepsin concentration closely parallels that of nitrogen concentration. Finally, a few cases of anacidity were reported in which, however, pepsin was still demonstrable.

In the previous communication the general range of pepsin output was indicated but not enough cases had been studied to set actual standards. The present paper deals with an analysis of a larger series of observations and with the diagnostic implications derived therefrom.

MATERIAL AND METHODS

Forty determinations were made on thirty-six hospital patients. Of these, twenty-one showed no evidence of disease of the stomach and were essentially normal people, five had duodenal ulcer, one had gastric ulcer, five had cancer of the stomach, two had pernicious anemia, one had sprue and two had anacidity for which no explanation was found. The determinations were made by the method previously described (2) on samples of gastric juice collected over ten-minute periods after histamine stimulation. The results are recorded in terms of milligrams of edestin digested by 1 cc. of gastric juice.

RESULTS

Chart 1 shows the shape of the curves of pepsin concentration and of total output of pepsin per ten-minute period after histamine stimula-

tion in a normal person. We wish to emphasize the fact that concentration falls whereas total output increases at the height of secretion.

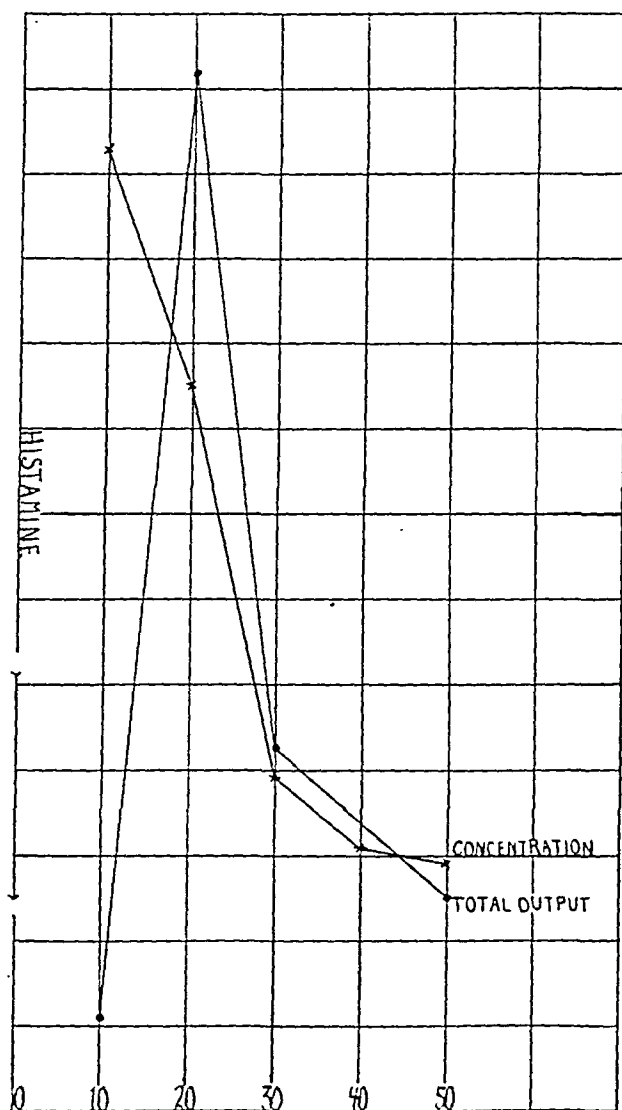


CHART 1. CURVES OF PEPSIN CONCENTRATION AND OF TOTAL OUTPUT OF PEPSIN AFTER HISTAMINE STIMULATION IN A NORMAL PERSON

It is evident, therefore, that in comparing the digestive power of gastric juice from various people the specimens which are tested must

TABLE 1
Pepsin output and other data concerning cases studied

Name	Diagnosis	Maximum 10-minute volume of secretion	Maxi- mum free HCl	Maxi- mum total acid	Concentra- tion of fer- ment at height of secretion (mgm. edestin digested by 1 cc.)	Total diges- tive power of 10-minute secretion (mgm. edestin)
		cc.				
Da	Unexplained anacidity	3	0		0	0
Sal	Carcinoma of stomach	5.5	0		21	118
La	Pernicious anemia	5.5	0		33	188
Ha	Pernicious anemia	4	0		85	240
Zi	Carcinoma of stomach	4	0		66	264
Sch	Carcinoma of stomach	5.5	0		57	285
Cr	Carcinoma of stomach	6	0		157	1,413
Gi	Unexplained anacidity	4	0		1,088	3,800
Ga	Sprue	17	60	70	272	4,624
Fi	Carcinoma of stomach	15	29	42	1,080	15,130
Al	Unexplained subacidity	16	11	32	1,325	15,900
Fl	Gastric ulcer	32	94	106	530	16,960
Mo	Normal	27	105	111	674	18,200
Ru	Normal	27.5	86	102	628	17,270
Ohr	Normal	19	126	138	860	18,540
Tal	Duodenal ulcer	55	128	133	350	19,200
Lord	Normal	26	135	145	750	19,500
Pu	Duodenal ulcer	45	142	148	462	20,800
Ros	Duodenal ulcer	38	135	140	570	21,680
Cl	Normal	25	66	79	1,164	23,280
Rob	Normal	38	122	125	660	25,100
Vi	Normal	32	117	124	899	28,780
Hu	Normal	44	97	102	668	29,400
Po	Normal	42	121	126	720	30,240
McI	Normal	26	107	116	1,242	32,292
Sc	Normal	47	63	75	853	34,400
We	Normal	30	82	96	1,204	36,100
McK	Normal	48	98	106	800	38,400
Ts	Normal	24	110	120	1,623	38,952
Hu	Normal	43	91	100	955	41,000
Pe	Normal	21.5	120	128	2,219	47,650
Ha	Normal	39	128	132	1,248	48,750
Wa	Normal	42	134	141	1,181	50,100
Jo	Duodenal ulcer	49	131	138	1,100	53,900
Sa	Normal	44	88	95	2,550	112,400
Val	Duodenal ulcer	84	140	146	2,500	210,000

represent similar points on the curve of secretion; it would obviously be useless to compare samples taken at random. In the present observations we have compared the digestive power of the total juice put out by various people during a ten-minute period at the height of secretion after a standard and powerful stimulus (histamine). This presumably gives the maximum rate of pepsin secretion of which the stomach in question is capable. The concentration of ferment (amount of edestin digested by 1 cc. of juice) was estimated on the same specimens.

The rate of pepsin secretion

Table 1 shows the cases arranged in series according to total output, i.e., rate of secretion of pepsin. The concentration of pepsin is also

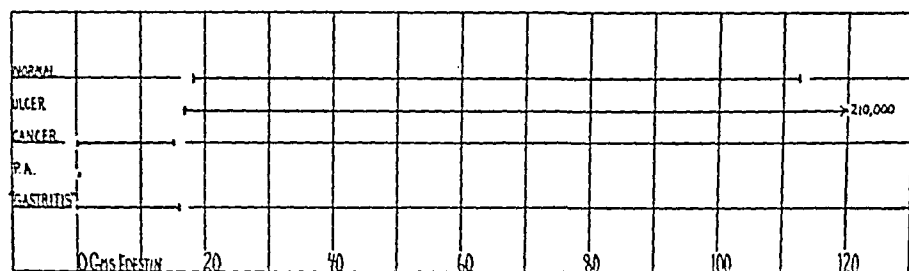


CHART 2. RANGE OF PEPSIN OUTPUT IN NORMALS AND IN PATIENTS WITH DISEASES OF THE STOMACH

recorded. A sharp line of demarcation can be drawn between the normals and the patients with ulcer on the one hand and those with diseases such as cancer, gastritis and pernicious anemia on the other. No normal person digested less than 16,000 mgm. with the maximum ten-minute output of gastric juice; no one with cancer, gastritis or pernicious anemia digested over 16,000 mgm. The relations are shown graphically in chart 2. The normal range, which coincides closely with that shown by the peptic ulcer cases, varied from 18,200 mgm. to 112,400 mgm. The extremely high output of pepsin in Val. (210,000 mgm.) is associated with the remarkable volume of secretion (84 cc.) which is nearly double the usual maximum. All but one of the juices from normals digested from 18,000 to 54,000 mgm. of edestin. Turning now to the abnormal cases with low pepsin outputs it is of

interest that in only one of eleven cases was it impossible to demonstrate the secretion of some pepsin even though the amount was small. Eight of these people failed to secrete free HCl (tested by dimethyl) even after histamine stimulation. It appears, then, that pepsin meas-

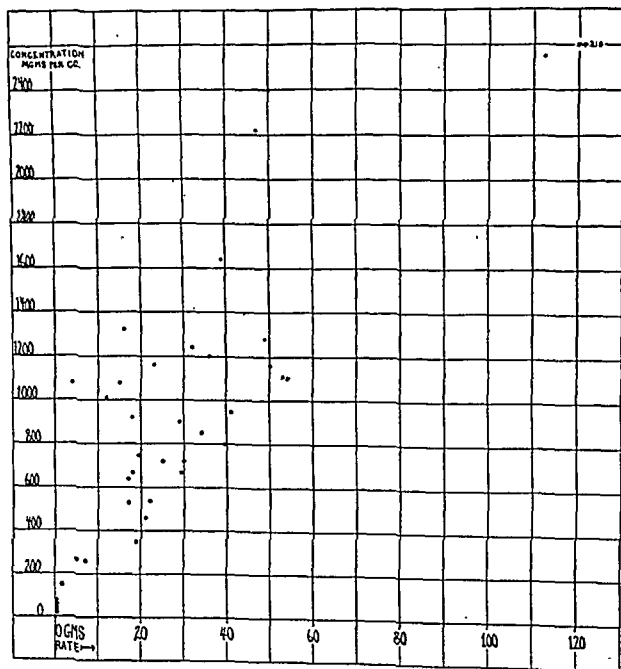


CHART 3. RELATION OF OUTPUT OF PEPSIN (RATE) TO CONCENTRATION OF PEPSIN

urements furnish a more delicate indication of deficiency of gastric secretion in some cases than determinations of acid, a consideration which may have some practical application.

The concentration of pepsin in the gastric juice

A consideration of the values for the concentration of pepsin set down in table 1 shows these figures to be of much less practical significance than the total rates of secretion. Both normal patients and those with advanced disease of the stomach showed high and low concentrations of pepsin; in only a few cases of cancer and gastritis were the values very much below those encountered in normals. There is, however, a general relationship between rate of pepsin secretion and concentration insofar as when the rate was great the concentration was usually high also (see chart 3).

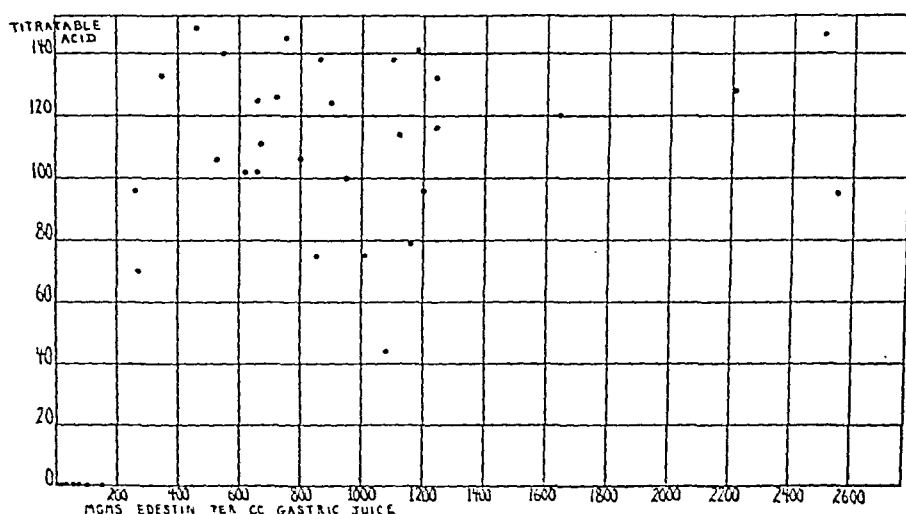


CHART 4. RELATION OF CONCENTRATION OF PEPSIN TO TITRATABLE ACIDITY

Relation of acidity to output of pepsin

A study of chart 4 in which the pepsin concentration is plotted against the titratable acidity reveals an interesting lack of correlation between the two. The cases with anacidity all showed low outputs of pepsin although the concentration of ferment was fairly high in some (see table 1), but it is evident that aside from such stomachs as are the seat of very extensive lesions pepsin is secreted without close relationship to acid. The whole subject of the secretory relationship of the various constituents of the gastric juice is not completely

understood as yet, but observations being made in our laboratory indicate that separate mechanisms control the secretion of water and of chloride as well as of ferment. The point is well illustrated by cases Al and Fi (table 1), who, with greatly reduced secretion of acid put out practically normal amounts of ferment. Gi, also, with no secretion of free HCl put out pepsin in normal concentration although the total quantity was low. The correlation of these relationships with exact histological studies would be of great value.

SUMMARY

The concentration and total output of pepsin have been studied in a series of normals and in people with various gastric lesions. It was found that in normals the maximum ten-minute volume of stomach juice digested from 16,900 mgm. to 112,400 mgm. of edestin. The ulcer cases fell within the same general range. In people with pernicious anemia, cancer of the stomach, and severe gastritis, the pepsin output was diminished, in some cases to extremely small amounts, but in only one person was pepsin completely undemonstrable. No correlation was found between pepsin output and acidity and it is suggested that the secretory mechanisms of the two are independent. Finally, it seems that very low pepsin output is a more delicate index of gastric damage than low acid values.

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OBSERVATIONS ON THE SURFACE CAPILLARIES IN MAN FOLLOWING CERVICOTHORACIC SYMPATHETIC GANGLIONECTOMY¹

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In the last decade, an impressive amount of evidence has accumulated to indicate that the capillaries are capable of changing their caliber as a response to nervous impulses, independently of the arterioles; that is, that their rôle under certain conditions is active rather than passive. Rouget, in 1873, was the first to present anatomic evidence of the presence of branched cells, which he assumed were contractile, on the endothelial walls of the capillaries. Vimtrup (1922), in Krogh's laboratories, has made histologic studies confirmatory of Rouget's work. The presence of contractile cells on the walls of capillaries has been shown in most tissues of mammals, also in birds, reptiles, and fishes, and more recently, in many tissues in man. Histologic proof is not complete, as contraction of capillaries has been demonstrated in the absence of Rouget cells. Clark and Clark (1925) recently reported that they have not been able to find contractile cells in the capillaries in tadpoles. Stöhr (1926) was only rarely able to demonstrate nerve fibers to the capillary endothelium. Anatomic researches by Vimtrup (1923) on diverse tissues of the human being, including the cutaneous capillaries and venules, have shown the existence of contractile or Rouget cells. Schaley has demonstrated their presence in tissues in the eye of the human being. Beale, in 1860, and Glaser, in 1920, demonstrated in some organs in certain mammals that the capillaries are accompanied by fine, nonmedullated nerve fibers paralleling the capillary. Their connection with the wall of the capillary also has been demonstrated in tissues from some regions.

¹ Read before the Central Society for Clinical Research, Chicago, November 22, 1929.

² With the technical assistance of Grace M. Roth.

The existence of vasomotor fibers to the cutaneous capillaries remains yet to be proved, but as Krogh stated, present histologic methods are probably not yet sufficiently refined to delineate such small sympathetic fibers. Krogh's classical researches on the physiology, and more especially on the number and size of capillaries, in a given unit of tissue has given further impetus to investigation of and information on their autonomous behavior. From the experimental standpoint, then, it can be stated that there is much anatomic and physiologic proof to indicate an independent capillariomotor mechanism, under control of vasomotor nerves, and subject to vasodilator and vasoconstrictor influence.

Several workers in this field, for instance, Kukulka (1920) and Redisch (1924), have advanced the theory that changes in caliber of the capillaries are due largely to chemical effects. This conception has been more extensively studied by Lewis and his associates. They believe there is a dilator substance present in the cells of the tissue, and that capillaries respond to certain forms of injury, including anoxemia and reactive hyperemia, in which there is liberation of a hypothetical dilating substance. These workers presented many convincing experiments and arguments. Krogh accepts this hypothesis, but advanced the opinion that more than one substance may be concerned in the reactions. Other investigators maintained that the change in the size of the capillary is due to variations in pressure dependent on the size of the arterioles. Kuntz (1929) recently summarized the subject by stating: "The data available at present do not afford an entirely adequate explanation of the caliber changes in the capillaries."

The effects of section of the sympathetic nerves and removal of the ganglions have been studied in the frog by several workers. The first effect is dilatation of the capillaries but the caliber is restored after a day, or restoration may be delayed for three months or longer. Even if dilatation of capillaries does not occur after sympathetic ganglionectomy, the state of the vessels is markedly modified, as shown by Drinker (1927) and Krogh, Harrop, and Rehberg (1922). They found that section of the sympathetic nerves produces prompt dilatation of the arteries and arterioles but that dilatation of capillaries is delayed for twenty to thirty minutes. Gabbe (1926) demonstrated in frogs an increase in the number and size of capillaries in muscle after

division of the sympathetic nerves. Closure of the main arteries, and elimination of the effects of pressure, did not prevent this response. Conversely, it has been shown by many workers that stimulation of the sympathetic nerves produces arterial and capillary contraction, and there is much suggestive evidence that the contraction of arterioles and of capillaries are separate and independent.

With the advent of operative measures in man, in which the sympathetic ganglions are removed, an exceptional opportunity has been afforded to study the effects of sympathetic ganglionectomy on the capillaries of the skin. It has been shown that following the removal of the cervicothoracic and second thoracic sympathetic ganglions in certain cases of Raynaud's disease, of thrombo-angiitis obliterans, and of scleroderma, dilatation of the arterioles of the skin takes place. This response has been measured by the increase which occurs in the surface temperature and by indirect methods for determining the volume of the flow of the blood.

The present study was to determine by microscopic methods whether demonstrable variations in the size and number of capillaries follows sympathetic ganglionectomy. Twenty-four cases in which cervicothoracic ganglionectomy was done by Adson and Craig are included in compiling this report. There were nine cases of Raynaud's disease; twelve cases of scleroderma, nine of which were of the vasomotor type; two cases of thrombo-angiitis obliterans in which there was a vasospastic disturbance, and one case of subacute arthritis, in which there were cold, moist extremities.

METHODS³

The capillaries of the nailfold were examined with the capillary microscope; measurements were made by an eye-piece micrometer; the rates of flow were determined by stopwatch, and by measuring the length of the visible capillary loop the rate of flow in millimeters for each second was calculated. Rates of flow in the capillaries, in excess of 1.5 mm. for each second, are impossible to measure, and rates in excess of this

³ The preoperative and postoperative observations in all cases were made on the same finger and on the same group of capillaries. Drawings were made for purposes of identification. The measurements of the width of the capillary represent the width of the column of blood in the capillary; the wall of the capillary is invisible.

are indicated as simply more than 1.5 mm. for each second. Determinations of surface temperature were carried out with thermocouples, a method embracing an accuracy of approximately $0.1^{\circ}\text{C}.$

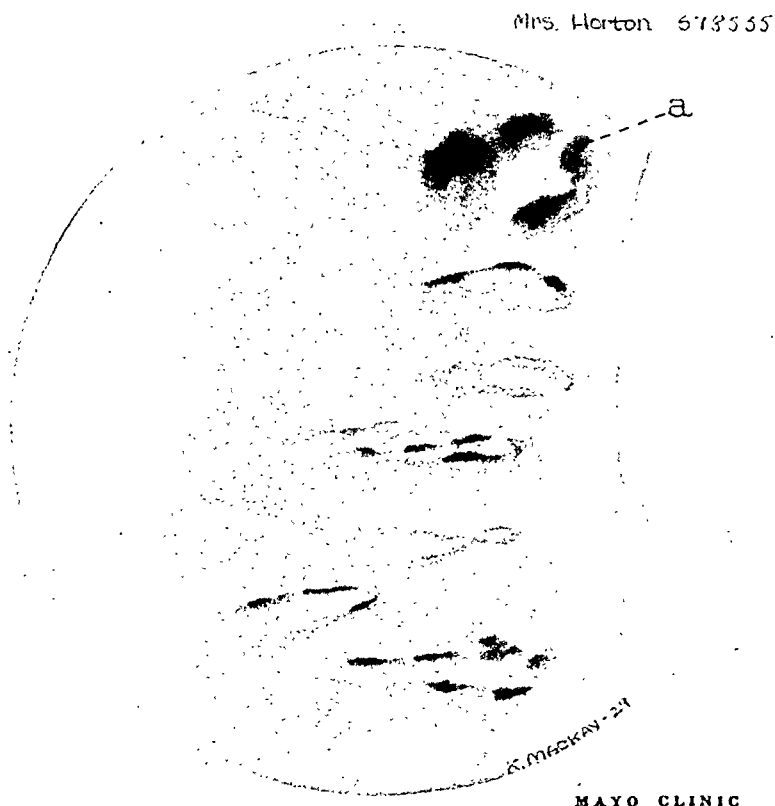


FIG. 1. CAPILLARIES OF THE NAILFOLD IN A CASE OF ADVANCED RAYNAUD'S DISEASE

The blood appears segmented. The loss of tone is evident in the marked dilatation of several capillaries.

Raynaud's disease

Raynaud's disease, in a well developed form, is characterized by attacks of pallor or cyanosis in the hands and feet, and occasionally in other acral areas. An attack usually is induced by cold, less frequently by psychic stimulation, and the attendant phenomena have a

symmetric distribution. The majority of cases occur in young females, usually of asthenic build and with other anatomic features of inferior constitution. The capillaries of the nailfold have been studied intensively in this disease by Parrisius (1921) and by Brown (1925). During the attacks of pallor the capillaries are incompletely filled, many loops are invisible, the flow of blood in the capillary loops ceases, and the blood which is visible has a cyanotic hue. The degree of pallor depends on the completeness of the constriction of the capillaries and the small venules. All gradations in pallor, varying from death-like colorlessness to mild grades of pale cyanosis are observed. The transition from the stage of pallor to cyanosis is frequently imperceptible. There is gradual but incomplete relaxation of the arterioles, which previously have been contracted. Segments of cells pass into capillary loops and frequently retrograde flow from the venules is observed; the capillaries slowly dilate and become distended with markedly cyanotic blood (fig. 1). More capillaries are opened. The stage of rubor, or recovery, which can be obtained by exposing the patient to elevated temperature, signalizes the relaxation of the arteriolar spasm, the resumption of rapid flow in the capillaries, and the transition of the cyanotic blood to blood of a bright red color; many capillaries become visible.

In spite of many observations, on a large number of cases of Raynaud's disease, it has been impossible to state decisively whether or not the independent contraction or dilatation of the capillaries and of arterioles is based on a neurogenic mechanism. However, there is much to suggest that this mechanism exists. This much is certain: in the cyanotic stage of Raynaud's disease, there is independent behavior of the arterioles and of the capillaries; the arterioles are somewhat contracted and the capillaries, dilated. It is a common clinical observation that in some subjects who have had attacks of Raynaud's disease for many years, recovery of the "normal" color of the skin between attacks becomes increasingly difficult, and eventually the hands become more or less permanently cyanotic. Mild degrees of puffiness, or a nonpitting form of edema may supervene. These observations suggest permanent impairment of tonus in the surface capillaries, which may be due to increased concentration of some vasodilating agent, perhaps a histamine-like substance, as

suggested by Lewis. The chemical factor apparently becomes predominant and maintenance of tonus of the capillaries is seriously and perhaps permanently impaired. In the arterioles there is increased tonus, as is shown by the fairly constant low temperatures of the skin. There is disassociation of behavior of the arterioles and of the capillaries.

There is every reason to believe that the basis of the vascular disturbance in Raynaud's disease is due to increased stimulation of the sympathetic apparatus. This is manifested not only in the behavior of the surface vessels, but also in the sweat glands, as evinced by local hyperhidrosis. Information on the neurogenic basis of the vascular spasm in Raynaud's disease is furnished by the frequent initiation of attacks by excitement and by psychic or emotional trauma. We have witnessed this in many instances; on our coming into the room to carry out venipuncture on these patients, attacks of pallor would be initiated before puncture of the skin had occurred. The recent work of Lewis (1929) seems to have thrown some doubt on the vasomotor basis of some cases of Raynaud's disease. He is inclined to think, from his experiments, that there is a variety of Raynaud's disease which is due to some abnormality of the muscle of the small arterioles. The evidence presented, however, is not convincing, for his procedure for interrupting the vasomotor fibers to the fingers was by blocking the peripheral nerves with procaine. Such interruption may be far from complete. In the present state of knowledge, it is impossible to say that all of the sympathetic fibers are conveyed to the periphery through the spinal nerves. Many fibers may be conducted along the adventitial sheaths of the arteries. That this is probably true is borne out by the fact that occasionally the improvement obtained in cases of Raynaud's disease by perivascular sympathetic neurectomy is only partial. The most convincing evidence concerning the vasomotor basis of Raynaud's disease is the complete cessation of spasm in the surface vessels of the feet following lumbar ganglionectomy. Many of these cases have been followed over periods of three or more years.

In the advanced cases of Raynaud's disease, changes in the capillaries, following cervicothoracic ganglionectomy, were fairly typical (table 1, fig. 2). There was decrease in the caliber of the capillaries in every instance, although moderate degrees of dilatation still persisted. The average decrease was from 0.07 to 0.03 mm. for the group. A

TABLE 1

Observations on surface capillaries before and after sympathetic ganglionectomy in cases of Raynaud's disease

Case	Age and sex	Caliber of visible capillaries (average)		Open loops in each square millimeter of surface area		Flow in capillary loop, millimeter for each second		Surface temperature		Comment
		Before	After	Before	After	Before	After	Before	After	
		mm.	mm.					°C.	°C.	
1	30 F.	0.08	0.05	5-10	16-18	0.09	>1.5	23.6	27.8	History of trouble for nine years, pallor, cyanosis, pain, minute dry ulcers
2	33 F.	0.08	0.03	8-10	12-14	0.019	0.20	22.8	30.7	History of trouble for seventeen years, coldness, cyanosis, pain, hyperesthesia, no ulcers
3	20 F.	0.05	0.02	10-12	20-22	0.004	0.57	23.2	32.9	History of trouble for four years, pallor, cyanosis, pain in hands, no ulcers
4	40 F.	0.07	0.03	5-6	14-16	0.06	0.33	23.7	30.3	History of trouble for six years, pallor, cyanosis, ulcers, pain in fingers of both hands
5	26 F.	0.08	0.05	5-6	16-18	0.05	>1.5	20.6	29.1	History of trouble for seven years; attacks of extreme cyanosis of hands and feet, pain, ulcers, advanced case
6	25 F.	0.08	0.03	10	15	0.005	>1.5	27.4	30.7	Advanced case, cutaneous ulcers, pain, recovery of color difficult to obtain
7	31 F.	0.05	0.02	8-10	12-15	0.005	>1.5	24.9	34.1	History of trouble for eighteen months, blanching, numbness, pain in fingers of both hands
8	30 F.	0.05	0.02	9	12-15	0.09	>1.5	30.5	32.6	History of trouble for seven years, color-changes, ulcers, early scleroderma
9	35 F.	0.10	0.05	6-9	11-13	0.002	0.10	24.0	28.6	History of trouble for two years, color-changes, pain, three ulcers of fingers with early sclerodermal changes in distal parts of fingers

significant increase took place in the number of visible loops for each square millimeter of surface area of skin. The number of loops in

different given areas of skin, before operation, varied from five to ten, and after operation, from twelve to twenty. This increase was noted in every case. There was definite change in the flow in the capillary loops. Instead of the broken, segmented, halting type of flow, the

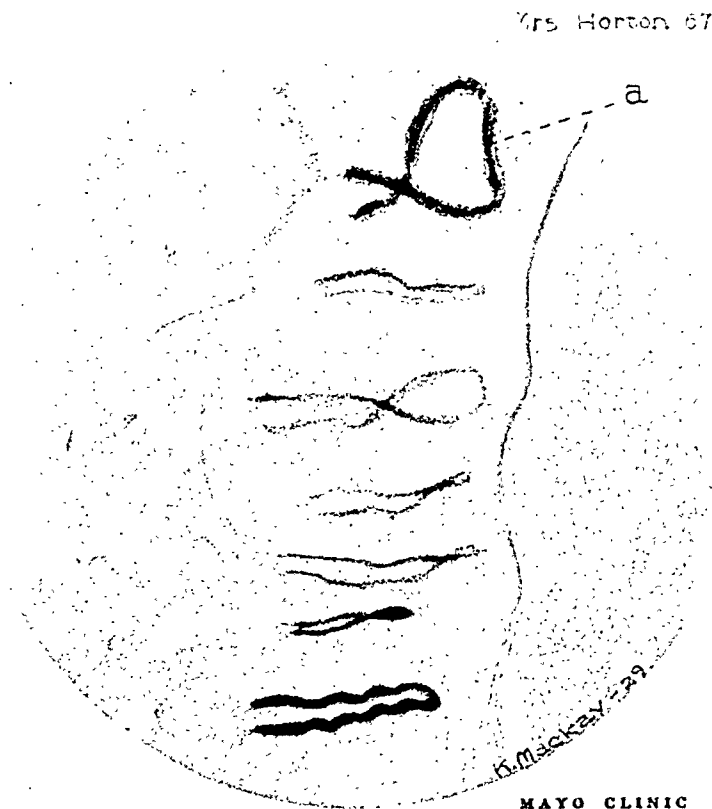


FIG. 2. CAPILLARIES OF THE NAILFOLD AFTER SYMPATHETIC GANGLIONECTOMY.
SAME GROUP AS IN FIGURE 1

There is narrowing of the visible capillaries. More capillaries are visible. The flow is rapid and regular.

flow was accelerated. Interruption and segmentation of the flow was unusual, and the color of the capillary blood at room temperature was bright red. The average value for the rates of flow in the capillaries for the group increased from 0.03 to a rate in excess of 0.95 mm. for each

second. When these patients were exposed to lowered degrees of local and environmental temperature (15 to 20°C.) the capillary flow was distinctly retarded. However, complete stasis, such as that which occurs with the slightest decrease in environmental temperature in cases in which operation is not done, was not observed. The increased surface temperature which occurs uniformly in these cases following operation and which averaged 6.2°C. for the entire group studied, indicates conclusively dilatation of the arterioles. The subcapillary venules, which before operation were distended and intensely blue and contributed largely to the cyanotic color of the skin, became invisible.

Scleroderma

This group represents eight distinct and selected cases of scleroderma four of which were characterized by an early history of all the characteristic phenomena of Raynaud's disease. Attacks of pallor and cyanosis in the extremities, induced by cold, have been the rule. Following a variable period, the skin has become thickened, motion of the fingers has become impaired, and, as the disease has advanced, pigmentation, atrophy, and trophic changes have appeared. These changes have affected the hands in the greatest degree, and usually in lesser degree the arms, face, back, legs, and feet. The vasomotor phenomenon usually has continued to be present during the stage of the development of the scleroderma. Chronic cyanosis of the extremities, such as that seen in Raynaud's disease, has been absent. Recovery from the attacks of spasm has been the rule. The studies of capillaries in this group of cases have been carried out by Brown and O'Leary (1925) and they confirm the opinion held by Cassirer (1912), by Leriche and Fontaine (1927) and by others, namely, that there is a form of scleroderma which is probably secondary to vasomotor disturbances of the type seen in Raynaud's disease.

I have observed that the so-called clinical condition designated scleroderma is a cutaneous disturbance secondary to many diseases, and that it occurs in cases of thrombo-angiitis obliterans, chronic arthritis, Raynaud's disease, and dermatomyositis. There is, no doubt, a primary form of scleroderma; four such cases were included in this series.

Examination of the cutaneous capillaries in the cases of scleroderma can be summarized. (1) There was a sharp diminution of the number of open capillaries for each unit area of skin. (2) Large, distorted, irregular, dilated capillary loops were present, the so-called giant capillaries. (3) Sharp slowing of the flow with slight change in the

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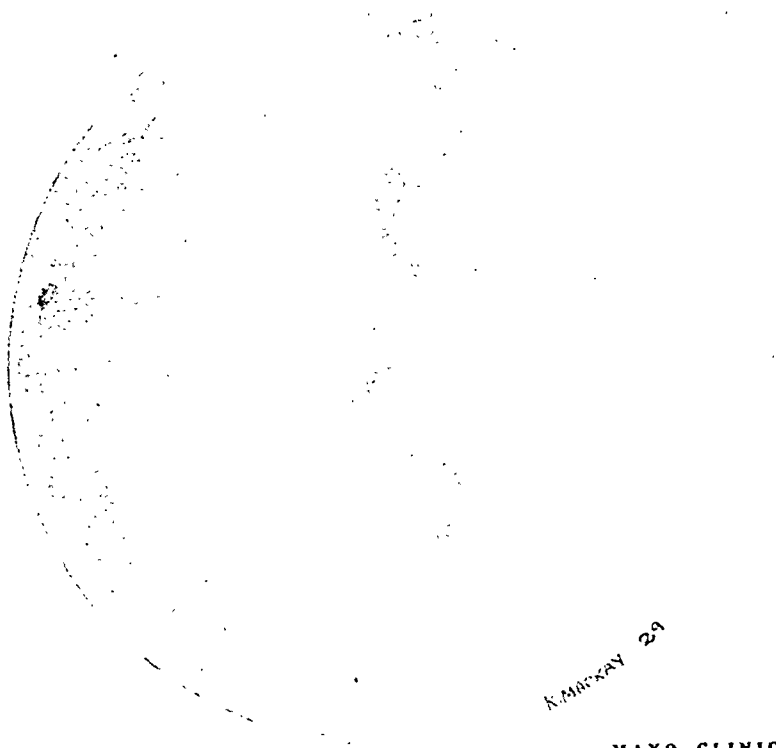


FIG. 3. CAPILLARIES OF THE NAILFOLD IN A CASE OF SCLERODERMA

The capillary loop is large and distorted. There is marked decrease in the number of visible loops.

environmental temperature took place. Stasis, with cyanotic capillary blood or irregular, intermittent flow could be induced easily by minor degrees of cold. (4) There was marked impairment in the transparency of the skin, which made impossible, with the best forms of illumination, clear definition of outlines of the capillaries; many of

TABLE 2

Observations on surface capillaries before and after sympathetic ganglionectomy in cases of scleroderma

Case	Age and sex	Caliber of visible capillaries (average)		Open loops in each square millimeter of surface area		Flow in capillary loop, millimeter for each second		Surface temperature		Comment
		Before	After	Before	After	Before	After	Be-fore	After	
		mm.	mm.					°C.	°C.	
10	36 F.	0.08	0.03	5-6	7-8	0.11	0.23	22.4	27.6	Marked absorption of terminal phalanges
11	37 M.	0.07	0.03	5-6	8-10	0.05	0.14	25.2	29.3	Primary scleroderma, ulcers of finger tips, early changes in face
12	57 F.	*	0.04	3-4	3-4	0.09	†	27.8	32.4	Advanced scleroderma hands and face, fixation of joints of hands, pigmentation, slight vasomotor disturbance
13	19 F.	*	0.03	3-4	4-6	*	0.28	25.1	29.0	History of trouble for nineteen years, vasomotor disturbance early in disease, advanced scleroderma of hands and face
14	30 M.	0.10	0.04	4-6	8-14	0.05	0.15	23.0	30.8	History of trouble for two years, primary scleroderma, trophic ulcers over knuckles, early involvement of face
15	41 M.	*	0.03	1-2	6	‡	‡	24.0	32.4	Primary scleroderma of hands, late vasomotor disturbances, pigmentation
16	56 F.	0.12	0.04	4-5	7-9	‡	‡	26.0	33.4	History of trouble for eighteen months, hardening of skin of hands, vasomotor disturbances consecutive with trouble, scleroderma fairly generalized, pigmentation marked
17	44 F.	0.08	0.03	4-5	7-8	0.03	‡	23.5	27.5	History of scleroderma for two years, consecutive fairly generalized vasomotor disturbance

* Impossible to determine.

† More than 1.5.

‡ Slow and indistinct.

TABLE 2—*Concluded*

Case	Age and sex	Caliber of visible capillaries (average)		Open loops in each square millimeter of surface area		Flow in capillary loop, millimeter for each second		Surface temperature		Comment
		Before	After	Before	After	Before	After	Before	After	
		mm.	mm.					°C.	°C.	
18	24 M.	0.20	0.06	2-3	3-4	‡	†	27.4		History of trouble for fifteen years, vasomotor disturbance of hands and feet, gradually developing sclerodermal changes
19	34 M.	0.07	0.04	5-6	7-8	0.07	†	25.6	30.6	History of trouble for eight years, vasomotor disturbance of hands and feet, gradual sclerodermal changes
20	37 F.	0.11	0.05	3-4	10	0.10	†	27.8	38.2	History of trouble for thirteen years, vasomotor disturbances of hands, scleroderma of the fingers and arms and slight involvement of skin over face and neck, small dry ulcers of finger and of feet, pigmentation, mild Raynaud's disease
21	50 F.	0.18	0.05	3-4	8-10	*	†	21.5	33.3	History of trouble for one year, early trophic ulcers of fingers, partial fixation of joints, vasomotor disturbances

the capillaries were seen as indefinite masses of erythrocytes. This diminished transparency of the skin was a fairly constant finding in the more advanced cases.

After operation several changes were noted (table 2, fig. 4). (1) There was definite narrowing of the lumen of the capillaries, averaging a decrease from 0.11 to 0.05 mm. (2) The number of open capillaries for each square millimeter of skin was increased approximately 50 per cent. (3) The average rate of flow in the capillaries increased from 0.07 to 1.05 mm. for each second. Stasis was not observed under controlled room temperatures, and the segmented or broken, interrupted type of flow, indicating arteriolar spasm, was much less striking or it had entirely disappeared. The capillary blood was red instead of

cyanotic, and (4) marked improvement in the visibility of the skin has been observed within five days after operation. The outlines of the capillary loops were clearly made out, and their contours could be traced easily, and there was definite change in the form of the loops,

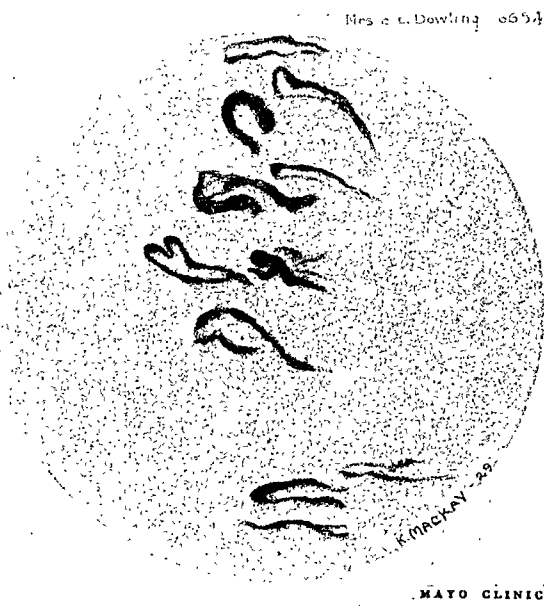


FIG. 4. SAME GROUP OF CAPILLARIES AS THAT IN FIGURE 3 AFTER SYMPATHETIC GANGLIONECTOMY

There is an increase in the number of loops and a narrowing of caliber of the capillaries. The capillary flow is rapid and uniform. Marked increase in the transparency of the capillaries is seen.

from an indefinite mass to a bizarre shaped loop with more clearly defined outlines.

It was apparent that the overactive sympathetic vasomotor effects on the arterioles were diminished, as in the uncomplicated cases of

Raynaud's disease, but not to the same degree. The changes in the transparency of the skin was of the greatest interest. Two possible factors may be responsible for this (1) With the acceleration of the flow in the capillaries and the probable improvement in permeability of the capillaries and metabolism of tissue, certain products or fluids may be removed from the cells in the skin, and (2) the brownish pigmentation which is so commonly present may gradually diminish after operation, thus altering the transparency of the skin.

The surface temperatures were increased after operation in every case. The increase was gradual during the first week after operation. The increase, however, was not of the same magnitude as that which occurred in the uncomplicated cases of Raynaud's disease, and averaged 5.6°C . for the group. This is easily understood when it is realized that in scleroderma there is organic quantitative diminution in the number of the cutaneous vessels and that new vessels do not open up in the short period of postoperative observation to the same degree as in Raynaud's disease. The increase in the surface circulation was due to the removal of the overactive vasoconstriction of the arterioles and the increase in the number of open capillaries.

Thrombo-angiitis obliterans

Two cases of thrombo-angiitis obliterans involving the larger arteries of the upper extremities were studied. Associated with the occlusive disease of the arteries was a vasospastic disturbance of the fingers, characterized by attacks of pallor and cyanosis following exposure to lowered temperatures. One or more of the palpable arteries of the wrist were occluded. The spastic disturbances were of great interest. They were undoubtedly secondary and due to the irritative inflammatory disease involving all coats of the arteries. Cases of thrombo-angiitis obliterans, with vasomotor disturbances, are very often erroneously diagnosed as Raynaud's disease in the male, as has been noted by Buerger (1924) and by Allen and Brown (1927).

The capillaries of the nailfold in the cases of thrombo-angiitis obliterans, with a secondary vasospastic disturbance, were essentially different from those in cases of Raynaud's disease (fig. 5). The capillaries were less dilated, there was a larger number for each unit area of skin, and the disturbances of flow were not as pronounced or as easily

induced with cold as in the cases of Raynaud's disease. Intermittency of the flow, and complete stasis, was not the rule at room temperature. With lowered environmental temperature, mild grades of stasis of the capillary flow were observed, many loops becoming invisible, and the

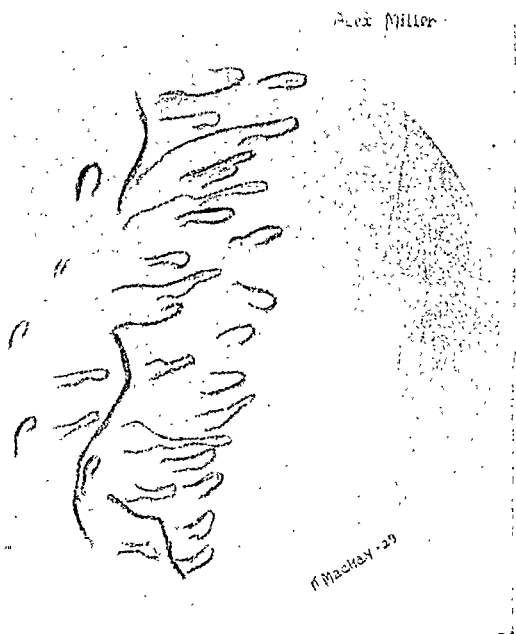


FIG. 5. CAPILLARIES OF THE NAILFOLD IN A CASE OF THROMBO-ANGIITIS OB-LITERANS AFFECTING THE HANDS

There is slight dilatation of the capillaries and venules

visible blood becoming cyanotic. Large, dilated, atonic capillaries, such as those observed at room temperature in well developed cases of Raynaud's disease were not observed. The tonus of the capillaries seemed to be maintained better than in Raynaud's disease. Following cervicothoracic ganglionectomy, the surface temperature of

the fingers and hands were increased, indicating arteriolar dilatation. The surface capillaries were increased in number and for each unit area of skin (fig. 6). The rates of flow in the capillaries were probably accelerated but they could not be measured accurately. With exposure to

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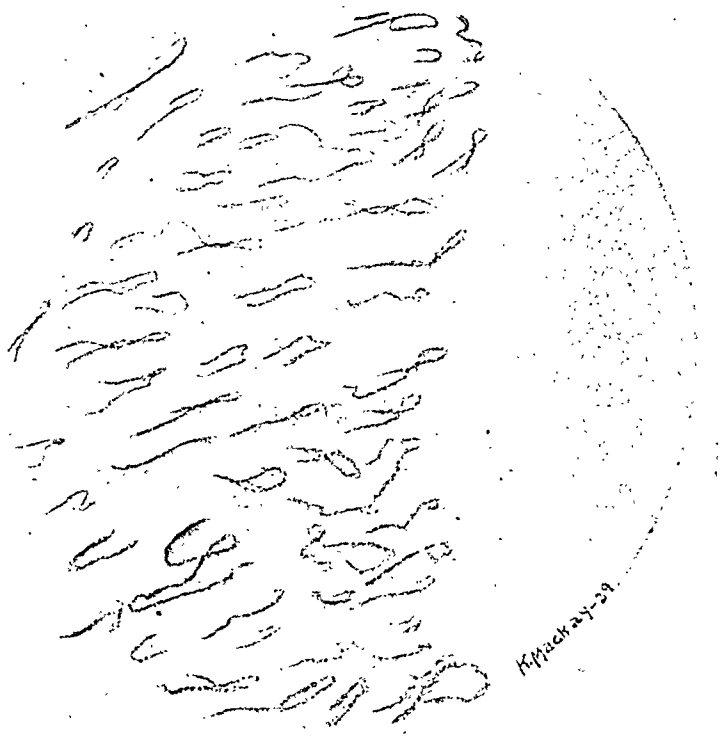


FIG. 6. SAME GROUP OF CAPILLARIES AS IN FIGURE 5

There is a marked increase in the number of visible capillaries and a slight lessening of the caliber.

lowered environmental temperature, stasis and intermittency of flow was much less noticeable. The changes in the surface vessels correlated well with the clinical improvement observed in these cases. Attacks of cyanosis and pallor disappeared, and there was a greater tendency of the hands to maintain a normal pink color. The rubor of the hands, while in the dependent position, was much less marked.

The changes that occur in the capillaries in cases of thrombo-angiitis obliterans are similar to, but less marked than, those which occur in Raynaud's disease. The surface temperature increases sharply immediately after operation. This must be due to the decrease in the exaggerated vasoconstrictor tonus of the arterioles of the collateral circulation.

TABLE 3

Observations on surface capillaries before and after sympathetic ganglionectomy in cases of arthritis and thrombo-angiitis obliterans

Case	Age and sex	Caliber of visible capillaries (average)		Open loops in each square millimeter of surface area		Flow in capillary loop, millimeter for each second		Surface temperature		PREOPERATIVE CONDITION
		Before	After	Before	After	Before	After	Before	After	
		mm.	mm.					°C.	°C.	
22	37 F.	0.03	0.03	14-16	16-18	>1.5	>1.5	23.3	33.6	Subacute arthritis, cold, clammy hands
23	35 M.	0.03	0.03	18-20	28-30	>1.5	>1.5	25.6	33.0	Numbness for three months, whiteness and cyanosis of fingers of right hand, continual pain, ulcer on first finger, thrombo-angiitis obliterans
24	34 M.	0.03	0.03	18-20	30-32	0.18	0.21	26.7	32.6	Vasospastic disturbance in hands, radial and ulnar arteries occluded, thrombo-angiitis obliterans

Arthritis

The capillaries in one case of subacute arthritis have been studied before and after cervicothoracic ganglionectomy (table 3). The hands in many of these cases were cool, moist and mildly cyanotic. Excessive sweating of the hands and feet is the rule. There were no significant variations in the size and form of the capillaries of the nail-fold, and there was a normal number for the unit area of skin. The capillary flow was easily retarded by chilling. These observations constituted evidence of overstimulation of the sympathetic apparatus. In many respects the appearance of the capillaries of the nailfold was that observed in the so-called normal persons with moist, cool extremities.

The only significant change noted in the capillaries after operation was an increased number of capillaries for each unit area of skin. There was no measurable increase or decrease in the caliber of the capillaries. No variation in the flow in the capillaries could be measured, both the preoperative and postoperative rates of flow being too rapid for measurement at room temperature. With lower environmental temperatures, fairly rapid rates of capillary flow were maintained after operation.

These observations in one case of subacute arthritis are contrary to what one would expect. Significant changes in the width of the capillaries could not be demonstrated. This was borne out by the normal pink appearance of the hands after operation. The significant effect of the operation, is the increased surface temperature of the hands.

DISCUSSION

Removal of the sympathetic ganglions in man does not induce dilatation of the cutaneous capillaries in the extremities of the patients who have been studied. In cases of Raynaud's disease there is loss of tonus of the surface capillaries, and their power of contraction is lost or greatly impaired later in the disease. In this disease there is loss of coördinate action between the arterioles and surface capillaries and venules. There is hypertonus of the arterioles, which is believed to be due to excessive stimulation of the sympathetic nerves, and there is hypotonus in the capillaries and venules which can be explained on a chemical basis. Sympathetic ganglionectomy in man exerts its greatest effects on the arterioles. This can be graded by measuring the sharp and continued elevation of the surface temperatures and by determining the rate of elimination of heat. The color of the normal skin is due largely to the number and size of the surface capillaries and venules and to the color of the contained capillary blood. Cyanotic color of the skin indicates increased exposure of capillary blood containing an increased amount of reduced hemoglobin. The normal pink color of skin would signify a lesser number of capillaries, narrower lumens and blood with oxygen saturation more closely arterial than venous. It follows, therefore, that when the sympathetic vasomotor nerves are interrupted, and the skin changes from a cyanotic to a red

or pink hue, there must be two reactions in the surface vessels: lessened exposure of capillary blood, either by lessening of the number of open vessels or decrease in their size, and change in the color of the capillary blood by increased rate of flow and resultant changes in its content of oxygen. After operation in Raynaud's disease there is a measurable degree of narrowing of the capillaries, and acceleration of the capillary flow. These effects, we believe, are due to dilatation of the arterioles, with more rapid flow of blood through the capillaries. This causes changes in the chemical environment and probably removal from the tissues of the hypothetic dilator substance. Crawford (1929) in a recent study, has shown that following experimental passive congestion in the capillaries of the ear of the rabbit, there is failure of these vessels to contract from sympathetic stimulation. Crawford advanced the opinion that the accumulation of some dilator substance, as suggested by Ebbecke (1923), Lewis (1929) and others, modifies capillary response. Krogh has noted that reactive hyperemia is induced not only when the flow of blood to a limb is entirely occluded, but also when it is reduced, as occurs in venous congestion. Lewis has shown that dilatation of the cutaneous vessels is active and is not due to changes in pressure. Such dilatation occurs in Raynaud's disease, which simulates venous congestion in the surface vessels. Many explanations have been offered for this phenomenon of dilatation, but none seems as convincing as that advanced by Lewis that there is accumulation of a dilator substance. The fact that the effects of sympathetic ganglionectomy on the capillaries and arterioles are opposed can be explained, I believe, only by a course of reasoning similar to that just explained.

Following sympathetic ganglionectomy, in cases of vasomotor forms of scleroderma, the capillaries are decisively changed. There is a decrease of 50 per cent in the width of the column of capillary blood. There still remains some dilatation, however, for the average width after operation is 0.05 mm., the normal being approximately 0.02 mm. The number of loops is increased, but not comparably to the increase in the cases of Raynaud's disease. This is due to the fact that in scleroderma there is a true organic decrease in the number of surface vessels. In four to six weeks after operation higher surface temperatures are present than one week after operation. This suggests that new vessels

gradually have opened. The improvement in the transparency of the skin is definite, and probably is an effect of improved lymphatic circulation with removal of fluids or other metabolic products from the skin. There is reason to believe that dilator substances play a part in these forms of scleroderma, but the number of capillaries is so reduced that their dilatation does not markedly affect the color of the skin. It seems likely that there is an additional factor in that the giant forms of capillaries, with marked distortion, represent compensatory efforts to develop a collateral circulation in the skin. This may explain the residual dilatation which remains after operation.

In the one case of arthritis, in which the patient could be considered a control or normal subject, the capillaries were of fairly normal tonus. After operation, no change in the caliber of the capillaries was found, increase in the number of loops was not noted, and the color of the skin likewise did not change. The sole quantitative vascular change was that of arteriolar dilatation as measured by the increase in the surface temperature and in the rate of elimination of heat. These observations on capillaries of normal or increased tonus, which has been subsequently verified by similar observations in two other cases, indicate that in the skin of the human being the abolishment of sympathetic innervation seems to have no visible or measurable effect on the size and number of the capillaries. In this case, the color of the skin resumed a more normal pink, which was due to increased rapidity in the capillary flow.

The impression gained by these studies is that in certain states of disease, in which the tonus of the capillaries was diminished, that of the arterioles was increased. Removal of the sympathetic ganglions has a paradoxical effect. There, tonus of capillaries is increased, and tonus of arterioles is decreased. The former effect can be explained on a chemical basis and the latter on the basis of direct response from dividing the vasomotor nerves.

SUMMARY AND CONCLUSIONS

Quantitative studies on the capillaries of the skin of human beings have been made both before and after cervicothoracic sympathetic ganglionectomy. Nine cases of advanced Raynaud's disease, four cases of vasomotor forms of scleroderma, four cases of primary

scleroderma, two cases of thrombo-angiitis obliterans, and one case of arthritis of the hands have been studied.

The operation did not cause dilatation of the capillaries (the usual physiologic effect) in any case. On the contrary, there was consistent narrowing of the dilated atonic capillaries in Raynaud's disease, and an increase in the number of visible capillaries. This could be explained on the basis of a diminished concentration of a theoretic chemical dilator substance in the tissues.

Following sympathetic ganglionectomy in eight cases of vasomotor and primary forms of scleroderma, there was definite reduction in the caliber of the capillaries. The number of open capillaries for each square millimeter of skin increased. There was quantitative evidence of arteriolar dilatation and lessened response on the part of these vessels to cold. Clinical improvement of some degree occurred in every case.

In the cases of thrombo-angiitis obliterans and arthritis, in which the capillaries were of fairly normal caliber and tonus, following operation no change in the width of the capillaries was noted. The intermittent slow flow of cyanotic capillary blood changed to one that was rapid, regular, and of normal color. The number of open capillaries was increased.

The major effect of sympathetic ganglionectomy is exerted on the arterioles. There is a sharp, maintained increase in the surface temperature of the skin of the hand in every case. These studies add some confirmation to the opinions of those who hold to the importance of chemical factors in modifying tonus in the capillaries of the skin of human beings. Chemical control of these vessels seems, under certain conditions, more effective than nervous control.

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ICTERUS NEONATORUM

III. THE OXYGEN CAPACITY AND SATURATION OF THE MOTHER AND FOETUS¹

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In our first communication on this subject (1) we concluded that "Icterus neonatorum is a physiologic condition, which is the result of a post-natal readjustment from an environment requiring the presence of polycythaemia, for the maintenance of oxygenation, to one in which no such extraordinary measures are necessary. Icterus is present in all infants, visibility being only a matter of degree."

In our second study (2) we succeeded in producing polycythaemia, and later icterus, in guinea pigs, after keeping them in a chamber under reduced atmospheric pressure. Very soon after the animals were removed from the low pressure chamber they all showed positive indirect Van den Bergh reactions, and increased icteric indices. These findings, just as in new born infants, coincided with the reduction of the polycythaemia. We had thus succeeded in producing experimentally, in animals, an icterus in which the antecedent causes were analogous to the conditions under which, in our opinion, icterus neonatorum appeared.

We had some evidence to warrant the assumption that the foetus lived in a condition of relative oxygen unsaturation. The polycythaemia itself, the large number of immature red cells, and the anatomy of the foetal circulation in which the bulk of the circulating blood is mixed, venous and arterial, all pointed towards this conclusion. We felt, therefore, that in order to complete our chain of evidence, it was essential to study the oxygen capacity and saturation in the mother and the foetus.

¹ This research was made possible through the kind generosity of Mr. Ernest Rossiter.

Studies of this nature have already been made in goats by Huggett (3). He has shown that the oxygen saturation of the arterial blood of the goat foetus is much lower than that of the mother. No human studies have ever been made.

We first studied twenty cases in which the labour was normal, or required only slight obstetrical aid, such as low forceps. In none of the cases studied did the child require any special methods for the initiation of pulmonary respiration. In addition, studies were made in five cases of cesarean section. The oxygen capacity and saturation were estimated by the Van Slyke method (4). The foetal blood was

TABLE 1
Patients delivered with chloroform anaesthesia

Case number	Foetus		Mother		Anaesthetic	Mode of delivery
	O ₂ capacity	O ₂ saturation	O ₂ capacity	O ₂ saturation		
	<i>volumes per cent</i>	<i>per cent</i>	<i>volumes per cent</i>	<i>per cent</i>		
2	22.82	78	19.56	91	Chloroform	Normal
5	21.34	76	18.92	93	Chloroform	Normal
7	22.23	70	18.56	89	Chloroform	Normal
9	21.92	79	18.35	93	Chloroform	Normal
11	22.35	79	18.79	93	Chloroform	Normal
12	21.82	70	19.24	89	Chloroform	Low forceps
13	22.34	71	17.53	88	Chloroform	Low forceps
14	20.87	82	18.03	93	Chloroform	Normal
15	21.56	79	18.21	92	Chloroform	Normal
18	20.83	70	18.76	89	Chloroform	Low forceps
19	21.73	71	19.92	89	Chloroform	Low forceps

taken from the umbilical vein, under oil, at the moment of clamping the umbilical cord. A tightly fitting needle on an all glass syringe was introduced into the umbilical vein. The barrel of the syringe contained a few potassium oxalate crystals, and a small quantity of oil. Estimations were made immediately afterwards. Maternal blood was taken from the radial artery, also under oil.

The twenty-five cases were divided into three groups. Group I, (table 1) comprised eleven cases in which chloroform anaesthesia was used. Of these eleven cases, seven were normal deliveries, and in four, low forceps were used. Group 2 (table 2) consists of five ces-

arean sections, in which nitrous oxide and oxygen was the anaesthetic used, and group 3 (table 3) represents nine cases in which neither anaesthetic nor other aids were required.

In all twenty-five cases, the oxygen saturation of the blood was distinctly lower in the foetus. The maternal figures were practically

TABLE 2
Patients delivered by Cesarean section with nitrous oxide—oxygen anaesthesia

Case number	Foetus		Mother		Anaesthetic	Mode of delivery
	O ₂ capacity	O ₂ saturation	O ₂ capacity	O ₂ saturation		
	<i>volumes per cent</i>	<i>per cent</i>	<i>volumes per cent</i>	<i>per cent</i>		
21	22.86	81	18.53	93	Gas and oxygen	Cesarean section
22	21.57	78	18.21	94	Gas and oxygen	Cesarean section
23	21.98	80	19.04	94	Gas and oxygen	Cesarean section
24	22.56	80	18.05	95	Gas and oxygen	Cesarean section
25	21.83	78	17.93	94	Gas and oxygen	Cesarean section

TABLE 3
Patients with unaided delivery, no anaesthetic

Case number	Foetus		Mother		Anaesthetic	Mode of delivery
	O ₂ capacity	O ₂ saturation	O ₂ capacity	O ₂ saturation		
	<i>volumes per cent</i>	<i>per cent</i>	<i>volumes per cent</i>	<i>per cent</i>		
1	21.72	81.1	19.34	94	None	Normal
3	20.89	79	18.1	94	None	Normal
4	22.12	80	17.95	95	None	Normal
6	22.32	76	18.12	94	None	Normal
8	20.82	78	18.92	95	None	Normal
10	22.85	82	17.89	94	None	Normal
16	20.98	80	19.21	95	None	Normal
17	21.34	79	19.34	94	None	Normal
20	20.93	81	18.96	94	None	Normal

normal, though lowest in the four cases requiring low forceps. They were slightly lower in the whole group receiving chloroform than in the other two groups. The oxygen capacity was greater in foetal than in maternal blood, in all twenty-five cases, obviously because of the polycythaemia.

COMMENT

In a previous communication we have drawn attention to the admixture of blood from the umbilical vein with the general venous circulation. This admixture takes place in the liver and heart. (Alpha factor of Lundsgaard and Van Slyke (5).

In the light of the experiments presented here, it now becomes apparent that the oxygen exchange in the placenta itself is defective. The placenta is a relatively poor respiratory organ as compared to the post-natal lung. (This is analagous to the "L" factor of Lundsgaard and Van Slyke.) How much of this deficiency of oxygenation of the umbilical vein blood can be accounted for by placental metabolism has not yet been determined.

CONCLUSIONS

1. Foetal arterial blood (umbilical vein) possesses an increased oxygen capacity and a diminished oxygen saturation.

2. This diminution in oxygen saturation of the blood in the umbilical vein is to be accounted for by the deficient respiratory function of the placenta.

3. Maternal blood from the radial artery shows values for capacity and saturation which are within normal limits.

4. Further evidence is thus adduced in support of our theory that the polycythaemia in the newborn infant is the result of oxygen unsaturation of the foetus in utero.

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THE FUNCTIONAL PATHOLOGY OF HYPER-PARATHYROIDISM

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For many years a few physicians have conceived of a condition the direct opposite of parathyroid tetany. Lundborg (53) and later Chvostek (21) believed this was manifest in myasthenia gravis but clinical observations did not confirm their belief. It was not until the use of Collip's (23) active parathyroid extract in animals had indicated the symptoms of such a state that hyperparathyroidism was recognizable as a clinical syndrome. It is now known that it constitutes a picture of disease as clearly defined as hyperthyroidism and follows closely the abnormalities which may be produced by parathyroid extract in animals. The literature and clinical features have been discussed in another paper (2).

Many facts concerning functional abnormalities in hyperparathyroidism have already been determined.

Collip (23) has shown that the various well-known manifestations following removal of the parathyroid gland are reversed after the parenteral administration of parathyroid extract. No longer is the theory tenable that the fall in serum calcium after parathyroidectomy is but a secondary phenomenon. Active extracts will prevent it and by raising the serum calcium entirely relieve the symptoms. Extracts readily cause an increase in the serum calcium in normal animals. It seems obvious now that the parathyroid glands are concerned with the regulation of the calcium concentration in the blood. They appear as an essential component in one of those remarkable regulatory mechanisms characteristic of the higher animals. The constancy of the serum calcium even under many of the most abnormal conditions is worthy of special emphasis. Although an increase in serum phosphate is typical of parathyroid tetany a reduction has not commonly been found in experiments in which parathyroid intoxication was rapidly produced. Robinson, Huffman and Burt (62), however, report a reduction in the serum phosphate of calves receiving parathyroid extract. Greenwald and Gross

(39) and later other investigators have definitely shown that effective parenteral administration of parathyroid extract causes an increased excretion and especially an increased urinary excretion of both calcium and phosphorus, phenomena quite the reverse of those following parathyroidectomy.

A change in the tone and irritability of muscles which might be considered the opposite of tetany is suggested in the hypotonia often noted in animals with hypercalcemia. Berman (9) using galvanic currents has demonstrated a definite decrease in the electrical response in the peroneal nerve of normal dogs after a moderate increase of serum calcium produced by parathormone. These results were emphasized by the contrast with his parathyroidectomized dogs.

Another noteworthy result of administering excessive amounts of parathyroid extracts, is the deposition of calcium in various tissues. This has been described by Hueper (48) and occurs characteristically in the lungs, gastric mucosa and kidneys. As one might predict from the negative calcium balance, long continued administration of parathyroid extract results in decalcification of the bones, a phenomenon which has been studied from the histological point of view by Bauer, Aub and Albright (3).

In the clinical literature one finds many cases of extensive bone diseases complicated by parathyroid tumors or hyperplasia. These cases are now recognizable as probable examples of hyperparathyroidism. The predominating type of bone disease was that characterized by multiple cyst and giant cell tumor formation and usually classified as *ostitis fibrosa cystica*. Less frequently other types of bone diseases, such as *osteomalacia*, metastatic carcinoma or multiple myeloma, revealed this association. Many of these cases with enlarged parathyroids displayed certain interesting features probably attributable to excessive parathyroid activity. General decalcification, fragility and bowing of the bones were usually evident. A prominent symptom in some cases was extreme muscle weakness. Many had kidney stones with *pyelonephritis* and *cystitis*. In some instances there was calcium deposition in various tissues, characteristically in the lungs, gastric mucosa and kidneys. The cases presented a variety of other symptoms possibly related to hypercalcemia, such as marked constipation, attacks of severe abdominal pain and vomiting as well as symptoms suggesting cardiac insufficiency.

Studies of calcium and phosphate metabolism in the cases of the older literature were infrequent and the doubt which often surrounds the diagnosis makes them generally inapplicable to the study of functional pathology in hyperparathyroidism. Since 1926, however, considerable data has accumulated. Mandl (56) who was the first clinician to search for abnormal parathyroid tissue with therapeutic possibilities in mind studied the urinary excretion of calcium before and after removal of a parathyroid tumor. Before the operation excessive amounts of calcium appeared in the urine and the excretion fell below normal after the operation. Gold (37) has reported a case with *ostitis fibrosa*, a parathyroid tumor and moderate hypercalcemia. Extirpation of the parathyroid tumor was attended with marked improvement and resulted in a definite fall of the serum calcium and of the calcium excreted in the urine. Hannon, Shorr, McClellan and DuBois (44) and later

Bauer, Albright and Aub (44) have studied a patient with a generalized bone disease characterized by decalcification and tumor formation. This patient showed a hypercalcemia, hypophosphatemia, a negative calcium balance and excessive excretion of calcium in the urine. After removing two apparently normal parathyroids the patient improved, although there was little change in the calcium and phosphorus metabolism. Belden (5) has described a case of a woman with a mottled decalcification of the bones without tumor formation and presenting other symptoms characteristic of this group. The serum calcium was 15.2 to 18.8 mgm. per 100 cc., the serum phosphate normal. Operation was attempted but no parathyroid tissue obtained. Duken (30) has described two cases of generalized bone disease with high serum calcium and in one a tumor in the region of one parathyroid gland.

While the studies reported here were in progress, Wilder (79) reported detailed observation of a patient with marked muscle weakness, rarefaction and giant cell tumors of bones and a palpable parathyroid tumor. There was a moderate increase in serum calcium, a definite decrease in serum phosphate and excessive excretion of calcium in the urine. Following the resection of the parathyroid tumor, the serum calcium fell almost to the tetany level and calcium all but disappeared from the urine. The patient improved markedly. Beck (4) removed two parathyroid tumors from a patient with *ostitis fibrosa*. The patient developed extreme tetany and died on the twentieth day after operation. Recently a case was reported by Boyd, Milgram and Stearns (13) as hyperparathyroidism whose symptoms and associations may be considered classical. The serum calcium was high, the phosphate within normal limits. After removing the parathyroid tumor there was a marked fall in the serum and urinary calcium and the calcium balance became strongly positive. Snapper (73) records a case showing similar changes in the serum and urinary calcium after operation. Hunter (49) has described another typical case which had an excessive excretion of calcium both in the urine and the stools.

The following study of the functional pathology of the disease was started in April 1928, and has been continued to the present time. It will indicate the abnormalities encountered and will emphasize factors to be considered in diagnosis and treatment.

METHODS OF STUDY

The patients with hyperparathyroidism were studied in the Metabolism Division of Barnes Hospital. Their diets were prepared in the special kitchen of the division. The food and fluid intake was under the supervision of a trained dietitian, assisted by nurses especially assigned to the individual cases. At least two persons checked the foods as well as the dietary calculations. Refused food was weighed

and the proper adjustment made at once. Calcium and phosphorus intake was calculated, with few exceptions, from the analysis of foods collected from the literature by Sherman (69). It was obviously necessary to make analysis of some foods. The same type of bread was always used and analysed repeatedly. Water from the city supply was given and the calcium from this source calculated from the analyses furnished by the St. Louis Water Works. This amounted to only a few milligrams a day and could have been neglected without serious error.

The stool and urine collections were under the supervision of the head nurse of the division, who is well trained in the management of metabolic studies. The stools were collected in four-day periods, being marked off by carmine taken with the breakfast with the beginning of each period. Because of the possible difficulties from constipation, all patients received liquid petrolatum and granulated agar-agar with each meal. The urine was collected in 12 hour periods and combined into the four-day periods.

The calcium in the urine was determined by the method of Shohl and Pedley (70), that of the feces by the method of Corley and Dennis (24). The total phosphorus of the urine was determined by the method of Fiske and Subbarow (33) after complete oxidation with sulphuric and nitric acids. Care was taken to expel finally all the nitric acid and at the same time not to continue the heating to dryness. This same method was employed for the total phosphorus of feces by using an aliquot from the filtered digested stool mixture of the Corley and Dennis fecal calcium method. Serum calcium was determined by the Clark and Collip (22) modification of the Kramer-Tisdall method; serum phosphorus by the method of Benedict and Theis (6). The serum was always separated from the cells within two hours after the blood was obtained.

CASES STUDIED

Three patients with hyperparathyroidism were studied. Their histories are only briefly summarized as they have been reported in considerable detail in another paper (2).

Case 1 was a farmer's wife, 58 years old, with generalized *ostitis fibrosa cystica*. Her past history was noteworthy in that early in life she developed a striking

dislike for certain foods, especially milk, and it is reasonable to believe her calcium intake was deficient throughout life. For about 20 years she had had urinary symptoms attributed to "inflammation of the bladder." For nearly ten years she had been slowly developing difficulty in walking until finally she was quite unable to support herself. About two years before this study was started, she developed, following trauma, a fusiform swelling of the first phalanx of the right index finger. This proved to be a bone tumor containing many giant cells. Rather slight trauma caused a fracture of a clavicle and later a humerus. Following the extraction of a tooth a tumor developed in one maxilla. A tumor also appeared on the shaft of the left ulna which showed on biopsy many giant cells similar to the tumor of her finger. Physical examination revealed a remarkable degree of muscle hypotonia. Electrical tests showed no response of the muscles to faradic stimulation. There was a marked kyphosis and moderate bowing of the thighs. The tumor of the left maxilla caused marked asymmetry of the face. The teeth were quite loose. Urine examination showed evidence of inflammation of the urinary tract. X-ray revealed marked decalcification of all the bones and irregular areas of rarefaction suggesting tumor formation. Flat plates of the kidneys showed a large collection of stones in both pelves. Examination of the blood showed a slight anemia, negative Wassermann reaction, normal nonprotein nitrogen, calcium 16 mgm., and phosphorus 1.4 mgm. per 100 cc. Later a tumor the size of a small walnut was discovered rising from behind the inner end of the left clavicle as the patient swallowed. Histological examination after removal showed this to be parathyroid tissue. The course following operation is described in detail in a later section.

Case 2 was a farmer, 38 years old, with multiple epulis. Several years before admission he had had, because of gastric ulcer, two operations, the second of which was followed by complete relief. There was no history of any dietary abnormality, he had always liked milk and frequently drank as many as five glasses at a meal. There was no evidence of muscle hypotonia and there had been no urinary disturbances. About 8 months before admission to the hospital he noticed what he called a "gumboil" of the upper jaw. It was at no time painful, but gradually became larger. Three teeth at the site of the lesion became loose and were extracted by the patient. On examination he was well nourished. The muscles were well developed and of good tone. In place of the left upper canine and the first and second bicuspid teeth a tumor was present which extended into the hard palate posteriorly and beyond the alveolar margin anteriorly. X-ray disclosed a smaller tumor of the opposite maxilla and three separate and distinct tumors of the lower jaw. The urine was normal. There was no anemia. The blood Wassermann was negative and the blood nonprotein nitrogen was normal. The phenolsulphonephthalein excretion was 55 per cent in two hours.

At two operations all of the tumors were removed by curette and cautery and treated with radium and deep x-ray therapy. Microscopic examination of the

tissue showed characteristic giant cell tumors. About six weeks after the operation on the jaw, the serum calcium and phosphorus were studied for the first time. Calcium values ranged from 13.3 to 16.7 mgm. and phosphorus from 1.6 to 2.9 mgm. per 100 cc. A more careful examination of the neck now revealed a small mass just above the inner end of the left clavicle. This was elevated and more easily felt when the patient swallowed. There was still no evidence of hypotonicity of the muscles. X-ray of the skull and long bones showed no other bone tumors and no decalcification. X-ray of the urinary tract revealed no stones. The course following the removal of the parathyroid tumor is described in a later section.

Case 3, was a housewife, 46 years old, with multiple myeloma, who had been ill for two and a half years with pain, weakness fever and night sweats. Clinically there was found to be a destructive involvement of vertebrae and ribs, enlarged and tender liver and kidney insufficiency. Values for nonprotein nitrogen ranged from 65 to 75 mgm. per 100 cc. and the phenolsulphonephthalein excretion was very low. No Bence-Jones protein was found in the urine, although albumin and casts were abundant. Serum calcium was found to be high and a study of phosphorus and calcium metabolism was started. A few days after the last studies reported in the text she developed broncho-pneumonia and died. The post-mortem examination demonstrated typical plasma cell multiple myeloma involving chiefly the vertebrae, pelvis and ribs. Three parathyroid glands were moderately enlarged. There was a remarkable degree of metastatic calcification of the lungs, gastric mucosa and kidneys.

TOTAL CALCIUM BALANCE

Sherman (68) after reviewing the literature offered what he considered to be a satisfactory estimate of the average amount of calcium required for maintenance of equilibrium. It appears that this should be, in normal adults, approximately 0.45 gram per 70 kilograms of body weight. All of our patients were studied for a considerable period, first while on a low calcium intake but on an intake well above the amount estimated by Sherman for equilibrium under normal conditions. In summary form, a part of the extensive data recorded at the end of the paper has been placed in table 2 to illustrate the type of calcium metabolism presented by these patients. There can be no doubt of the abnormality. Although their intake was theoretically adequate, they excreted excessive amounts. In each case, over one-half gram per day failed to keep them in balance. Even on a high intake, the first patient excreted more calcium than ingested; in Period 7 while taking 2.2 grams of calcium daily, the average output was 3.9

grams. The second patient barely maintained equilibrium on a very high intake and it seems probable that a negative balance might finally have ensued if the studies had been followed longer. The third patient who had generalized hyperplasia of the parathyroids and evident kidney insufficiency retained calcium for a long time when the intake was raised to about 1.3 gram daily, but here some unrecognized factors probably complicated the results because during these periods the calcium output was even less than in the former periods of low intake. The kidney insufficiency with its associated phosphate retention may have been of great importance. The probable significance of this factor will be discussed later. The clinical consequence of the failure to retain calcium is evident. Bone changes would be expected. Cases 1 and 3 showed marked generalized decalcification, a finding not infrequently associated with specific diseases of the skeleton.

URINARY CALCIUM EXCRETION

In 1884 Davies-Colley (27) described to the Pathological Society of London a case which was remarkably similar to Case 1 in this report. This was a girl 13 years old with generalized bone disease including a tumor of the jaw, nephrolithiasis and paraplegia. He stated that the urine "showed about one-third the amount of phosphate of normal, but the calcium in excess." Since then studies of the metabolism in bone disease have occasionally shown cases with an increased urinary output of calcium. The possible significance of the distribution of calcium excretion between the feces and urine has not been sufficiently emphasized in its relation to specific types of bone disease.

The most striking and noteworthy feature of the metabolism of the patients with hyperparathyroidism was the excessive excretion of calcium in the urine, corroborating exactly the experiments on animals with parathyroid extract. Normally the urinary excretion of calcium is relatively small and is not materially increased by high calcium intake. Even intravenous calcium administration may have little influence on urinary excretion (65). The great differences in output resulting from variations in calcium intake occur chiefly in the stools. It hardly seems necessary to review the literature concerning the excretion of calcium in the urine; one may refer to Givens' article (36) for a discussion of the older literature. The average quantity

excreted in twenty-four hours is usually 100 to 200 mgm. Frequently much less, and occasionally two or three times this amount has been recorded. One gets the impression that studies on active adults show a greater urinary calcium excretion than studies on inactive subjects with various diseases. This is also indicated by the data presented in

TABLE 1
Calcium excreted in the urine in 24 hours by hospital patients and normal individuals

Case number	Diagnosis	Urine calcium	Case number	Diagnosis	Urine calcium
		grams			grams
2	Arteriosclerotic heart disease	0.012	6	Diabetes insipidus	0.038
2	Arteriosclerotic heart disease	0.064	16	Diabetes insipidus	0.202
2	Arteriosclerotic heart disease	0.013	22	Diabetes mellitus	0.011
3	Arteriosclerotic heart disease	0.004	11	Diabetes mellitus	0.007
3	Arteriosclerotic heart disease	0.061	10	Diabetes mellitus	0.008
4	Arteriosclerotic heart disease	0.018	8	Diabetes mellitus	0.093
5	Arteriosclerotic heart disease	0.011	7	Diabetes mellitus	0.057
5	Arteriosclerotic heart disease	0.024	7	Diabetes mellitus	0.107
14	Arteriosclerotic heart disease	0.065	9	Diabetes mellitus	0.197
20	Arteriosclerotic heart disease	0.015	21	Streptothricosis of bone	0.012
27	Arteriosclerotic heart disease	0.005	30	Scleroderma	0.048
12	Arteriosclerotic heart disease	0.007	30	Scleroderma	0.080
23	Rheumatic heart disease	0.043	29	Spondylitis deformans	0.243
28	Rheumatic heart disease	0.013	29	Spondylitis deformans	0.329
28	Rheumatic heart disease	0.136	17	Mild acute nephritis	0.082
18	Pulmonary emphysema	0.049	31	Nephritis with edema	0.003
18	Pulmonary emphysema	0.081	31	Nephritis with edema	0.020
19	Lobar pneumonia	0.011	31	Aneurysm, bronchopneumonia	0.018
26	Hypernephroma	0.063	25	Normal	0.042
15	Carcinoma of stomach	0.054		Normal	0.045
24	Carcinoma of stomach	0.202		Normal	0.124
1	Carcinoma of liver; jaundice	0.018		Normal, high calcium intake	0.288
13	Diaphragmatic hernia	0.075		Normal, high calcium intake	0.312

table 1 showing the 24-hour urinary calcium excretion of hospital patients with various conditions and of a few normal individuals. The patients were taking regular ward diets and were allowed milk as desired. The data are also presented for comparison with the subjects having hyperparathyroidism. Acidosis especially when caused by mineral acids is associated with an increase in urinary calcium output.

Acid and basic diets produce similar changes. These facts are often cited but the studies of Zucker (83), Bogert and Kirkpatrick (11), and others indicate that the variations are not extreme. As the four adult subjects of Bogert and Kirkpatrick shifted from control diets to base forming diets the average urinary calcium excretion decreased from 99 to 66 mgm. in 24 hours, while 144 mgm. was excreted with

TABLE 2

Summary of calcium metabolism in hyperparathyroidism. The data are selected from tables 9, 10, and 11 and are expressed in daily averages in grams

Period number	Days	Calcium. Daily averages				Serum	
		Intake	Output	Urine	Stools	Calcium	Phosphorus
Case 1							
		grams	grams	grams	grams	mgm. per 100 cc.	mgm. per 100 cc.
1-5	20	0.56	1.36	0.319	1.04	16.4	1.5
6	4	0.91	0.72	0.780	1.94	16.5	
7	4	2.21	3.90	2.32	1.58	16.4	1.3
8	4	0.98	1.76	1.08	0.68	16.4	
Case 2							
1-4	16	0.89	1.08	0.432	0.65	16.8	1.6
5	4	3.69	3.40	0.587	2.81	14.9	1.9
6-7	8	2.33	2.46	0.532	1.93	14.0	2.1
9-10	12	0.99	1.22	0.451	0.77	14.1	2.4
Case 3							
1-4	16	0.56	1.15	0.246	0.90	15.4	2.6
5-8	16	1.37	0.91	0.144	0.77	15.7	5.3
9-10	8	3.58	2.15	0.226	1.92	17.8	4.8

acid-producing diets. Zucker found changes of a similar magnitude in men after hydrochloric acid and sodium bicarbonate. The various anions of physiological importance also appear to produce small but demonstrable alterations in urinary calcium excretion.

The data recorded in table 2 leave no doubt of the abnormal excretion of calcium in the urine of these patients with hyperparathyroidism. Even on a low calcium intake it is excessive. Especially impressive is

the output which ensued in Case 1 when the calcium intake was increased. In Period 7, the excretion amounted to 1.3 gram per day. The urinary output of Case 3 was less impressive but was greater than that of the patients included in table 1 who were studied under somewhat similar hospital regime. Here the evidence of kidney insufficiency complicated the picture and it seems reasonable to conclude that the abnormality was markedly limiting the capacity to excrete calcium by this route. Evidence is available indicating this effect. Halverson, Mohler and Bergeim (42) found a very low calcium excretion in a number of cases of nephritis. Administration of calcium causes only a slight increase. The same phenomena were also noted by Boyd, Courtney and MacLachlan (12) in children with nephritis and by Schriver (66) in two cases which were studied in great detail. In extensive observations of a case in this clinic the urinary calcium was found to be almost negligible. Hetenyi and Nogradi (45) showed that nephritics excreted less than normal subjects after intravenous injections of calcium salts.

Certain conclusions may be drawn from these studies regarding the relation of the urine reaction to this abnormal calcium excretion. The astonishing urinary calcium excretion of Case 1 was associated with a very acid urine. Under certain conditions to be discussed later, however, the urine calcium was subsequently reduced to normal (Period 16 to 19, table 9) at times when the urine was even more acid. The other patients produced urine which was nearly neutral or at times slightly alkaline. It seems obvious that the reaction of urine cannot be a factor of primary importance although an increase in acidity may exaggerate the tendency to abnormal excretion of calcium.

Hypercalcemia was found in all three cases and it is natural to assume a relationship between this and the excessive urinary calcium. Certain circumstances, however, indicated that the connection is more complicated than might at first appear. When excessive amounts of calcium were given the increase in urinary excretion was not necessarily attended by any further rise in the serum calcium. The most striking example of this was presented by Case 1 in Periods 7 and 8 (table 2). Very significant appears the fact that in Period 8 the great increase in urinary calcium excretion was associated with no further increase in hypercalcemia but with the lowest phosphate concentration which was

encountered in our studies. Calcium and phosphorus are so intimately related in body processes through their conspicuous compound, calcium phosphate, that it seems we are seldom justified in considering their functions and abnormalities separately.

FECAL CALCIUM EXCRETION

Bearing in mind that the rate of excretion of urinary constituents depends so largely on their concentration in the blood the excessive urinary calcium excretion of these patients might logically have been inferred. The influence of hypercalcemia on intestinal excretion would depend more on factors inherent in the functional activity of the intestinal epithelium and chemical reactions in the intestinal content. Greenwald and Gross (40) found an unmistakable increase in the fecal calcium excretion of dogs who were receiving parathyroid extract for several days. In other experiments (41) of about two months' duration the increase in fecal calcium was less marked. Hoag et al. (46) studied the influence of parathyroid extract on the calcium metabolism of seven infants, one normal, four rachitic and two with infantile tetany. Two rachitic infants showed a fairly definite increase in fecal calcium while receiving the extract; one with tetany, a definite decrease; while the others showed little or no variation from normal. Brehme and György (14) determined the effect of parathyroid extract on two infants. While the urinary excretion of calcium was markedly increased there was no change in the stool excretion. Stewart and Percival (75) during periods of a few hours found no increase in the rate of calcium excretion into the intestines of cats which had been given parathyroid extract. In calves receiving parathyroid extract, Robinson, Huffman and Burt (62) demonstrated a considerable increase in serum calcium but only a slight increase in urinary excretion and no decided change in fecal excretion. Shohl, Wakeman and Shorr (71) studied the calcium metabolism of two infants with tetany. Parathyroid extract did not alter the calcium excretion in the stools. After a very extensive investigation of the effect of parathyroid extract, on eight individuals, Albright, Bauer, Ropes and Aub (1) concluded that it had no influence on the fecal excretion of calcium. Hunter's case (49) of hyperparathyroidism had excessive amounts of calcium in the stools in addition to the increase in the urine.

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quate means of preventing ununited fracture in old age. Maxwell and Miles (58), and Vaughan (77), and especially Hutchison and Stapleton (50) have presented clinical evidence from which we may conclude that diminished muscular activity must be looked upon as of considerable importance as a causative factor in the development of osteomalacia. It is also interesting to note that Degkwitz (29) appears to have been able to prevent rickets in young dogs grown in the dark by giving them vigorous daily exercise.

The reaction of stools of Cases 1 and 3 was determined by the method of Tisdall and Brown (76). The pH ranged from 5.8 to 7.1, corresponding to that of patients with other conditions.

PHOSPHORUS METABOLISM IN HYPERPARATHYROIDISM

While in the literature on bone diseases much prominence has been given to changes in calcium metabolism, we must bear in mind the fact that one could as consistently put the same emphasis on the behavior of phosphorus for these two elements are inseparably involved in bone structure. A rôle of considerable importance has been assigned to phosphorus in the extensive investigation of rickets where defects in phosphorus metabolism have even been thought to be primary. There seems to be no good reason for denying that many of our apparent abnormalities of calcium utilization may be primarily disturbances of phosphorus metabolism. We have at present no certain knowledge whether the parathyroid glands or vitamin "D" or ultra violet radiation are concerned primarily with the physical chemical reactions of calcium or of phosphorus. From their experiences with human subjects, Albright, Bauer, Ropes and Aub (1) felt that the primary effects of parathormone was on phosphorus rather than on calcium. Greenwald and Gross (39) have stressed the marked change in phosphorous excretion following parathyroidectomy and parathormone injections.

A study of our three patients, however, has not presented us with any typical abnormality of phosphorus metabolism in hyperparathyroidism. It so happened that three cases presented distinct differences. Case 1 for 24 days (Periods 1 to 6, table 9) on a low and average calcium intake showed a definitely negative phosphorus balance excreting each day 300 to 400 mgm. more than she ingested.

The data showing the relative amounts of calcium excreted in the stools of three patients with hyperparathyroidism are summarized in table 2 together with the corresponding calcium intake and urinary output.

The fecal excretion in Cases 1 and 3 is quite impressive. Both patients on an intake which should have been adequate, excreted more calcium in the stools alone than they ingested. Case 1 for the first twenty days received in her diet an average of 0.56 gram of calcium daily. During this period she excreted by way of the intestinal tract an average of 1.04 gram daily. In the following period a daily oral intake of 0.91 gram of calcium resulted in the excretion of 1.94 gram in the stools. Thereafter on an excessive calcium intake the stool output did not exceed the intake. Case 3 gave a similar figure: for 16 days an average intake of 0.56 gram was accompanied by a stool output of 0.90 gram daily. The data are thus definite and leave no doubt that these two patients tended to have an excessive fecal calcium excretion. Case 2, however, presented quite a different picture. Clinically the case was typical of hyperparathyroidism with giant cell bone tumors, hypercalcemia, increased urinary calcium excretion and a tumor proved microscopically to be composed of parathyroid tissue. In no period of study was there any indication that the fecal excretion of calcium was excessive.

The possibility arises that some factor or factors other than hyperparathyroidism were operative in Cases 1 and 3 to produce increased excretion in the stools. One striking contrast existed between these patients and Case 2. They were bedridden and had been for months while Case 2 was up and about, moderately active and allowed to take daily walks in the vicinity of the hospital. It is not unlikely that the striking difference in activity of these patients may have had a most significant influence on their calcium metabolism. Bone must be looked upon as living tissue with an inherent capacity for adjustment to functional demands. We have no knowledge of the effects of inactivity on calcium and phosphorus metabolism but are well aware of the bone atrophy which may develop with disuse. Since it appears that most studies of calcium metabolism in disease have been made upon subjects who are quite inactive, this factor may have influenced the results. Further insight into the problem may suggest more ade-

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Following this on a high calcium intake, a slight amount of phosphorus was stored. Case 3 (table 11) with evident kidney insufficiency stored phosphorus while on a negative calcium balance. Case 2 (table 10) continually retained a slight amount of phosphorus; otherwise there was no evident abnormality of phosphorus metabolism. It seems almost paradoxical that he should, for such a long time, have retained phosphorus while continually losing calcium. McCrudden (54), and Miles and Feng (59) and others have noted a retention of phosphorus in

TABLE 3

Summary of the phosphorus metabolism of three patients with hyperparathyroidism. The data are selected from tables 9, 10, and 11 and are expressed in the daily averages in grams

	Period number	Serum		Phosphorus				Urine phosphorus Stool phosphorus	Comment
		Calcium	Phosphorus	Intake	Output	Urine	Stool		
		mgm. per 100 cc.	mgm. per 100 cc.	grams	grams	grams	grams		
Low calcium intake:									
Case 1.....	1-5	16.4	1.5	0.90	1.39	1.311	0.076	17.1	Bed-ridden
Case 2.....	1-4	16.8	1.6	1.42	1.24	0.976	0.269	3.6	Moderate activity
Case 3.....	1-4	15.4	2.6	0.81	0.71	0.380	0.330	1.2	Bed-ridden. Kidney insufficiency
High calcium intake:									
Case 1.....	7	16.4	1.3	0.90	0.80	0.785	0.020	39.2	Bed-ridden
Case 2.....	5	14.9	1.9	1.46	1.08	0.826	0.260	3.2	Moderate activity
Case 3.....	9, 10	17.8	4.8	0.99	0.68	0.200	0.480	0.4	Bed-ridden. Kidney insufficiency

the presence of a negative calcium balance. In this connection it is interesting to note that McCrudden found little variation from normal in phosphorus in the ash of bones of osteomalacia although the calcium was definitely reduced.

When the metabolism of phosphorus in these patients is examined in greater detail and the distribution of the excretion of phosphorus between the stool and urine is noted, interesting contrasts are manifest. The characteristic differences are illustrated by the data selected in table 3. Here are recorded the daily average phosphorus balances of

the three patients together with the phosphorus excreted in the stool and in the urine on a low and on a high calcium intake. The phosphorus metabolism of Case 1 is notable for the minute amount in the stool and the great quantity in the urine. Even when the calcium ingested was excessively high there was no increase in phosphorus in the stool. In fact, these periods exhibited the lowest stool excretion we have encountered. The great urinary excretion was no doubt related to some extent to the acidity of the urine, but it is questionable whether this was the sole basis for the unusual distribution of phosphorus between stool and urine. Evidence against this as an adequate explanation is the fact that in later periods (Periods 15 to 19) under different circumstances the urinary excretion was less when the urine was even more acid. A phenomenon worthy of special emphasis was the association of only minute quantities of phosphorus with the excessive fecal calcium. It is commonly assumed that calcium phosphate is precipitated in the gastrointestinal tract and that an excess of one element will increase the elimination of the other. This reaction may at times be very important. Briggs (15) has recently demonstrated to what extent calcium may increase fecal phosphorus excretion and has suggested the application of this action to the treatment of nephritis with phosphorus retention. However, the very significant studies of Bergeim (7) showed a surprising independence between these two elements in their absorption from the intestines. The findings just noted in Case 1, serve to emphasize the importance of Bergeim's work. It seems of interest to refer here to a case of true osteomalacia which we have recently studied. The phosphorus metabolism stood in marked contrast to that of Case 1. A large fecal calcium excretion was associated with an increased amount of phosphorus in the stools and a remarkably small quantity of phosphorus in the urine.

The distribution of the phosphorus excretion in Case 2 may be considered normal. The deviation from the normal in Case 3 was the reverse of the first case. Presumably because of the kidney insufficiency the urinary excretion of phosphorus was diminished. A more detailed study of the urinary excretion in table 11 indicates a progressive lowering of the ability of the kidney to excrete phosphorus. The stools show a moderate increase in phosphorus associated with excessive calcium output. The increase of both calcium and phosphorus

in the stools presents a picture simulating osteomalacia. This, together with the fact that the patient lacked exercise and vitamin "D," barely suggests we were dealing with such a complication. It might be noted that in the literature where phosphorus metabolism has been determined in that heterogeneous group of cases termed "*osteomalacia*" no constant changes were recorded; the calcium and phosphorus metabolism did not run parallel and in the majority of cases the phosphorus balance was positive.

In table 9 are recorded both the total and the inorganic phosphorus in the urine of Case 1. The discrepancy between the figures point to a large amount of organic phosphorus up to a time when orthophosphate was administered. Since this discrepancy was not observed in the other patients the inorganic phosphorus determinations were omitted from the tables. More recently determinations of organic phosphorus have been made by more accurate methods on a large number of patients with various diseases, including a fourth case of hyperparathyroidism (74). The amount of organic phosphorus in the urine was constantly quite small.

Experience in administering orthophosphate to patients with hyperparathyroidism was very instructive from a theoretical point of view and suggested important therapeutic possibilities. It seemed probable that if sufficient phosphate could be absorbed serum phosphate might be increased and serum calcium lowered. If this alteration could be accomplished it was predicted that the calcium metabolism might be restored to normal. In order to obtain maximal absorption of both elements, it seemed advisable to administer one at as remote a time as possible from the administration of the other and thus reduce to a minimum the precipitation of insoluble calcium orthophosphate. Therefore, 1 gram of sodium acid phosphate together with 3 grams sodium bicarbonate was given at 6:00 A.M., 10:30 A.M., 3:00 P.M., and double this amount at 10:00 P.M. while the calcium lactate was given three times each day with meals at 7:30 A.M., 11:30 A.M., and 5:00 P.M. The result of the procedure was quite striking. The data obtained from Case 1 is taken from table 9 and summarized in table 4. Period 8, preceding the first administration of phosphate, is recorded in the top row of figures for comparison. The results of this period were similar to the preceding periods but were complicated

by the fact that it followed a period of high calcium and during the period the patient received 8 grams of sodium bicarbonate daily. During Period 9, the patient received sodium acid phosphate four times each day in the manner described above. The following significant observations were made: (1) Almost all of the phosphate was absorbed for only a minute quantity appeared in the feces; (2) 70 per cent of the phosphorus ingested was excreted in the urine, and 27 per cent was retained in the body; (3) the serum phosphate rose to

TABLE 4

Data illustrating the influence of sodium orthophosphate per os on the abnormal calcium metabolism of hyperparathyroidism. The data are collected from table 9 and are expressed in daily averages in grams

Period number	Calcium				Phosphorus				Serum		Comment
	Intake	Output	Urine	Stool	Intake	Output	Urine	Stool	Calcium	Phosphorus	
	grams	grams	grams	grams	grams	grams	grams	grams	mgm. per 100 cc.	mgm. per 100 cc.	
8	0.98	1.77	1.085	0.68	0.90	0.85	0.84	0.01	16.7	1.3	NaHCO ₃ , 8 grams daily
9	0.97	1.34	0.195	1.15	2.07	1.52	1.49	0.03	12.5	3.8	Phosphate as described in text
10	0.98	1.96	1.625	0.33	0.86	1.02	0.60	0.42	12.4	1.4	NaHCO ₃ , 8 grams daily
11	1.19	0.48	0.112	0.37	2.05	1.09	0.87	0.23	12.8	5.9	Phosphate as described in text
12	1.21	0.97	0.142	0.83	2.08	1.48	1.14	0.34			Phosphate as described in text
13	1.18	1.47	0.107	1.36	2.10	1.86	1.18	0.68	12.9	3.0	Phosphate as described in text

normal; (4) the serum calcium fell to a level within the range of normal variations; (5) the urinary calcium excretion for the first time fell to normal; (6) a large amount of calcium appeared in the stools associated with almost no phosphorus. Period 10 intervening before further administration of phosphate served to emphasize the marked alterations which were produced. Here, the patient received sodium bicarbonate as in Period 8 but no phosphate. Conditions tended to revert to their former state. The following facts were evident: (1) There

was a negative phosphorus balance; (2) the serum phosphate fell again to an exceedingly low level; (3) the calcium balance was markedly negative; (4) there was a remarkable increase in the urinary calcium excretion; (5) this amazing output of calcium in the urine was associated with no increase in serum calcium. Attention was called above to the fact that the level of the serum phosphate may have an important influence on the excretion of calcium by the kidneys. The associations here attest the fact. Without any alterations in the serum calcium and while practically normal, the calcium excretion increased remarkably as the serum phosphate fell from normal to a very low level. In the next periods, 11, 12, and 13, phosphate was resumed. Again the serum phosphate rose and the urinary calcium excretion was controlled. This was not attended by excessive calcium in the stools until Period 3. So for the first time it was possible not only to maintain the patient on calcium equilibrium but to see her store appreciable amounts.

These results should be emphasized because of their possible therapeutic application. There is a rather extensive early literature on the effects of phosphorus in bone disease, and in a number of instances records of metabolic studies (47). But these seem in no way comparable to the results recorded here for they involve the administration of small doses, such as one or two milligrams of yellow phosphorus. There appeared to be no definite influence on calcium metabolism.

An apparently significant fact should be noted here. For one month (Periods 14 to 20, table 9) while other studies were in progress, there was no further administration of phosphate. Periods 14, 15, and 16 were used as controls and conditions were similar to the early periods of study. It would appear that the former phosphate administration produced a lasting change for the urinary calcium excretion remained low. This was associated with a continually negative phosphorus balance from excessive phosphorus in the stool. During these seven periods more phosphorus was excreted than had been stored during phosphate administration. In four periods the patient had stored 9.42 grams of phosphorus while in these seven periods she lost 18.49 grams. The serum phosphate remained normal. These periods again suggested the possibility that the level of the serum phosphate might be an important factor influencing urinary calcium excretion.

Urinary calcium remained low even though the serum calcium gradually rose to its former high level. There was a continually negative calcium balance due to the excess of calcium in the stool.

Periods 21, 22, 23, and 24 are recorded in table 9 for the sake of completeness. Sodium phosphate was administered as before. But unfortunately through a misunderstanding and in the absence of the authors the type of sodium orthophosphate given was not recorded. It is certain that during part of the time, perhaps throughout these periods, she received disodium orthophosphate. The outstanding feature of this series is the extremely large amount of calcium excreted in the stools. This may have been related to excessive ingestion of alkali. In Period 25 where the phosphate had been stopped and sodium bicarbonate given, the fecal calcium excretion was amazing. However, one might refer back to Periods 8 and 10 where alkali seemed to have the opposite effect, or Periods 19 and 20, where no influence on fecal excretion was evident.

Other instructive data showing possible effects of orthophosphate were presented during the study of Case 3. For eight days (Periods 9 and 10, table 11) on an exceedingly high calcium intake she stored considerable calcium. During this time the serum calcium rose to 17.8 mgm. per 100 cc. and with this there was a striking change in her general condition. She became stuporous and irrational. She developed râles at her lung bases, distended cervical veins and dependent edema, signs which were interpreted as circulatory failure. These prominent changes were thought to be directly related to the high serum calcium. Prompt reduction seemed imperative. This was accomplished by neutral sodium orthophosphate by mouth and attended by marked improvement. A solution of monosodium and disodium phosphate was prepared, the two proportioned to give a pH 7.2.¹ It was administered as in Case 1. The transition to her former state was most impressive. Within 24 hours she was alert again, quite rational and appeared stronger. In 48 hours the serum calcium had fallen to 11.7 and the serum phosphate risen to 8.6 mgm.

¹ NaH_2PO_4 (Merck C. P.) 12.8 grams and $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ (Baker) 138.7 grams dissolved in 500 cc. of distilled water gives a solution with a pH 7.2 and containing 0.3 gram of phosphorus in each 10 cc.

per 100 cc. The condition presented by this patient appears to be the same as that frequently seen with animals when the serum calcium has risen to a high level after parathyroid extract administration. Edwards and Page (32) particularly noted the depression of shock-like character with dulling of sensory reactions which occurred in some of their dogs.

THE EFFECT OF IRRADIATED ERGOSTEROL

Numerous investigators have shown that antirachitic vitamin promptly increases the intestinal absorption of both calcium and

TABLE 5

Data showing the results obtained when irradiated ergosterol was administered in hyperparathyroidism. The data are selected from table 11 and are expressed in the daily averages in grams

Period number	Calcium				Phosphorus				Comment
	Intake	Out-put	Urine	Stool	Intake	Out-put	Urine	Stool	
	grams	grams	grams	grams	grams	grams	grams	grams	
14	0.67	0.67	0.187	0.48	0.95	1.96	0.66	1.30	Control
15	0.67	1.16	0.250	0.91	0.97	2.48	0.64	1.84	Control
16	0.67	1.11	0.185	0.93	1.05	1.84	0.67	1.17	Control
17	0.67	0.73	0.082	0.65	0.87	1.31	0.71	0.60	Irradiated ergosterol
18	0.70	1.32	0.222	1.10	0.96	1.16	0.73	0.43	Irradiated ergosterol
19	0.69	0.97	0.142	0.83	0.88	1.00	0.66	0.34	NaHCO ₃ , 8 grams daily
20	0.69	0.99	0.140	0.85	0.89	1.54	0.59	0.95	NaHCO ₃ , 16 grams daily

phosphorus in experimental rickets and in infants with rickets. No such effect has been found in normal human adults. Wilder (79) reports improvement of his patient with hyperparathyroidism on a régime which included a high vitamin "D" intake. Metabolic studies of his patient during this period showed a slight retention of calcium and a more marked retention of phosphorus. An attempt was made in these studies to determine the effect of irradiated ergosterol on the calcium and phosphorus metabolism of hyperparathyroidism. The results were rather indefinite but seem worthy of presentation. Relevant data obtained from Case 1 are summarized in table 5. The three 4-day control periods followed directly after periods of high phosphorus administration, otherwise they were similar to the first periods of the

series. Irradiated ergosterol was given for only eight days. During periods 17 and 18 she received 18 drops of Acterol (Mead-Johnson and Company), each day. In Period 17, there was a slight drop in the calcium excretion which may have been unrelated to the irradiated ergosterol. The decrease in the total phosphorus in the stools appears more significant. This was associated with a moderate increase in urinary phosphorus excretion. The sodium bicarbonate in the two following periods seemed to have little influence on the calcium and phosphorus excreted. The experiment indicated a definite increase in the absorption of phosphorus by the gastrointestinal tract which appeared to be related to the irradiated ergosterol. The experiments of Warkany (78) are noteworthy in considering this point. After administering irradiated ergosterol to rachitic infants disodium-phosphate by mouth caused a great increase in serum phosphorus within two hours whereas previously it had had practically no effect.

THE EFFECT OF REMOVING PARATHYROID TUMORS

The most impressive experiences encountered in these studies were the amazing events after the first patient reverted from hyperparathyroidism to extreme tetany following the removal of a parathyroid tumor. They emphasize the relationship of the phenomena to parathyroid activities. On August 2, 1929, (the third day of Period 26) a tumor about 2.5 cm. in diameter was excised from the region of the lower pole of the left thyroid. Microscopically this proved to be parathyroid tissue. The chemical changes which followed are summarized in table 6. On the third day after operation the patient noted a change in her general condition, although the complaints were rather indefinite. There was a feeling of weakness and a sensation of tingling about the face and in the hands. There were obscure cramp-like pains in the extremities. Chvostek's sign could be demonstrated repeatedly. By the fourth day the serum phosphate was 2.2 mgm. and the serum calcium had fallen to 11.3 mgm. per 100 cc. By the seventh day the above symptoms had become exaggerated and she had become very much distressed and apprehensive. She had been nauseated and this day vomited. There was at times definite hyperventilation causing slight dyspnea. The numbness and tingling of the face and hands was marked. Twitching about the face was evident. The pain in

31	18th post-operative	200	18		5.0	3.0	3.02	1.15	0.006	1.14	0.50	0.24	0.10	0.14
	19th post-operative	125	18		5.1	3.2								
	20th post-operative	250	40		5.4									
	21st post-operative	100	22	1.7	5.5	3.7								
32	22nd post-operative	50	0	1.0										
	23rd post-operative	25	12	2.0	6.6	3.1	2.24	1.95	0.026	1.92	0.57	0.20	0.05	0.15
	24th post-operative	50	24	2.0	6.0	2.4								
	25th post-operative	50	24	2.0	6.8	2.3								
	26th post-operative	50	25	2.0	7.8									
33	27th post-operative	50	25	2.0	7.4	2.3	3.39	0.64	0.015	0.63	0.88	0.37	0.03	0.34
	28th post-operative	50	25	2.0										
	29th post-operative	50	25	2.0	7.4	1.7								
	30th post-operative	50	41	2.0										
	31st post-operative	50	25	2.0	8.5		3.88	1.05	0.035	1.02	0.88	0.15	0.02	0.13
34	32nd post-operative	50	25	2.0										
	33rd post-operative	50	25	2.0	8.7	2.5								
	34th post-operative	50	25											
	35th post-operative	50	25	2.0	9.1	2.5	3.45	0.63	0.008	0.62	0.87	0.27	0.03	0.24
	36th post-operative	50	25											
35	37th post-operative	50	25		9.7	2.3								

* Parathormone kindly furnished by Eli-Lilly and Company.

extremities continued. Chvostek's sign was present. Attempts to demonstrate Trousseau's sign caused marked tingling in the arms and forearms, but no definite contracture. The serum phosphate was 5.2 and calcium 10.8 mgm. per 100 cc. The patient had obvious tetany with normal serum calcium. The urine contained large quantities of ketone bodies and the serum CO_2 was 37 volumes per cent. This marked ketosis was unexpected. Although the food intake had been limited because of nausea, the ingested carbohydrate was quite enough to have prevented ketosis under ordinary conditions. Blood sugar determinations were normal.

On the ninth day the patient's distress appeared almost unbearable. The occasional attacks of vomiting continued. Trousseau's sign was no more definite than before. The patient described momentary attacks which indicated spasm of the larynx. She complained at times of inability to see. The eyelids drooped suggesting a paresis although they could be raised with voluntary effort. The serum phosphorus had risen to 11.3 and the calcium fallen to 7.4 mgm. The blood nonprotein nitrogen was 42 mgm. indicating the absence of any marked kidney insufficiency. The blood pressure was 132/80.

On the tenth day, at the beginning of Period 29 the patient started to receive calcium lactate by mouth in the amounts indicated in the table. After two days the only effect of calcium administration was a remarkable fall of serum phosphate to normal. This extreme alteration in serum phosphate had no influence whatever on the tetany which was becoming alarming. There was great pain in the muscles, which were not especially tense. The patient stated that the muscles had a sensation of worms crawling through them. No fibrillary twitchings could be seen. She now had a definite Trousseau's sign and occasional spontaneous contractures of muscles of the forearm were observed. There were also spasms of the extraocular muscles and short but definite attacks of laryngismus stridulus.

During the next four-day period (No. 30) the calcium lactate was increased and parathormone was given without any influence on the patient's condition. The serum calcium fell to 4.1. Vigorous forcing of the carbohydrate had cleared the ketosis. The following period was characterized by the fact that large amounts of calcium by mouth and parathormone administered subcutaneously and intravenously

did not alter the serum calcium. On the 18th day she received 40 grams of calcium lactate and 250 units of parathormone yet the next morning serum calcium was only 5.4 mgm. Tetany was not influenced until the 21st day when intravenous calcium chloride in relatively small doses was started. This caused prompt and dramatic alleviation of symptoms. On the morning of the 21st day after operation she was given 0.7 gram of calcium chloride intravenously and that evening 1.0 gram. The improvement was not noted immediately following the injections but occurred more gradually during the day. Twenty-four hours after the first injection she had received only 1.7 gram but was stronger, quite rational, and able to sit up in bed and eat breakfast without assistance. It is strange that with the marked improvement there was only a very slight increase in the serum calcium. For the next two weeks calcium chloride, two grams daily intravenously, was continued in addition to calcium lactate and parathormone. The serum calcium rose gradually to normal.

From this experience it was natural to conclude that the excessive quantity of calcium given by mouth was not absorbed, but examination of the data in table 6 shows that after parathormone was started large amounts of calcium were retained without its having any influence on the level of the serum calcium. During the eight days included in Periods 30 and 31, 13 grams of calcium were retained, while the tetany advanced in severity and a low serum calcium persisted. The regulation of serum calcium appears to be almost entirely independent of calcium absorption and to depend chiefly upon internal factors existing in the blood and other body fluids. The striking contrast between the effects of a little calcium chloride intravenously and a large quantity of calcium absorbed from the gastrointestinal tract is impressive but the reasons for this are quite obscure.

With the transformation which followed the resection of a parathyroid tumor there was a change in the urinary calcium excretion. For over a week it remained normal and then almost ceased. The change was so sudden as to suggest a kidney-threshold phenomenon. The calcium disappeared from the urine when the serum calcium had fallen to 7.5 mgm. per cent. Further studies, however, did not establish an absolute threshold, for later appreciable amounts of calcium were excreted by the kidneys in periods during which the serum cal-

cium was quite low. The remarkable fall in urinary calcium after operation was also observed by Mandl (56), Gold (37), Wilder (79), Hannon et al. (44), Boyd et al. (13), and Snapper (73).

Greenwald and Gross (39) noted in their experiments with dogs that the most striking change in metabolism after parathyroidectomy was a marked retention of phosphorus. This was not necessarily accompanied by any increase in serum phosphorus. They also found a decrease in the urinary phosphorus excretion. Changes in the phosphorus metabolism similar to Greenwald's were noted in Case 1 as she reverted to hypoparathyroidism following the removal of the parathyroid tumor. There was an immediate drop in the phosphorus excretion with the production of a positive balance. In comparison with the early periods of study (1 to 5) there was a slight increase in fecal excretion. The change to a positive balance was due to a decrease of phosphorus in the urine. This was at first associated with a remarkable increase in the serum phosphorus which reached the surprising figure of 11.3 mgm. per 100 cc. on the 9th day. It is noteworthy that there was an immediate fall in the serum phosphorus without modification of the urinary phosphorus when calcium was administered. The administration of parathormone at this time was not attended by any alteration in the phosphorus metabolism.

The study of the phosphorus and calcium metabolism of Case 1 was continued for over ten months following her operation. The interval was characterized by marked retention of both calcium and phosphorus. From the data we can summarize the calcium and phosphorus balance of the entire period as follows:

Total calcium intake.....	927.27
" " output.....	577.19
Calcium retained.....	350.08
Total phosphorus intake.....	311.67
" " output.....	131.76
Phosphorus retained.....	179.91

Calcium exists in bone chiefly as $\text{Ca}_3(\text{PO}_4)_2$ together with a small quantity as carbonate. If we calculate the amount of calcium which would combine with the 179 grams of phosphorus to form $\text{Ca}_3(\text{PO}_4)_2$ a

value of 348 grams is obtained to compare with the 350 grams of calcium actually stored.

For a year and a half following operation, Case 1 required an excessive calcium intake and parathormone to prevent tetany. The bones became rigid. The tumor of the maxilla receded. The teeth became tight. She gained considerable strength but the chronic urinary tract infection associated with bilateral nephrolithiasis continued in spite of treatment. A moderate retention of nitrogen gradually developed. Removal of the kidney stones seemed unjustifiable because of her poor general condition. She finally died fourteen months after the parathyroid tumor was removed, from uremia and the progressing urinary tract infection.

On June 18, 1929, at the beginning of Period 12 parathyroidectomy was performed on Case 2. A tumor about 3 cm. in diameter was removed under local anesthesia from the region of the left lower pole of the thyroid and behind the inner end of the clavicle. Microscopic examination proved this to be parathyroid though the section showed very little normal parathyroid tissue. The bulk of the tissue was made up of deeply stained cells with large nuclei with apparent attempts to form acinar arrangement. The day following operation the patient was able to be up and resume his usual activities. Twenty-four hours after operation the serum calcium had fallen to 10.6 mgm. per 100 cc. In 48 hours it had fallen to 9.7 mgm. with no change in the serum phosphorus. At this time, in response to questioning, he stated he had a little tingling about his face and fingers. Trousseau's or Chvostek's signs were not present. On the fifth day at a time when the serum calcium was 8.6 mgm. and phosphorus 3.7 the patient developed a slight diarrhea having four watery bowel movements during the morning accompanied by some abdominal cramps. There was nothing unusual in the diet that might account for this. The lowest serum calcium, 8.3 mgm. per 100 cc. was encountered on the 7th day; the serum phosphorus had increased to normal. There was no change whatever in his general body sensation. There was no evidence of any definite change in muscle tone. Chvostek's and Trousseau's signs had never been elicited. He had had some slight cramps in the calves of his legs at night but insisted this had been a common experience for years.

TABLE 7

Summary of the changes in the blood and in the calcium and phosphorus metabolism of Case 2 following extirpation of a parathyroid tumor

Period number	Day	Serum		Calcium. Daily average				Phosphorus. Daily average			
		Calcium	Phosphorus	Intake	Output	Urine	Stool	Intake	Output	Urine	Stool
		mgm. per 100 cc.	mgm. per 100 cc.	grams	grams	grams	grams	grams	grams	grams	grams
5	Pre-operative	14.9	1.9	3.69	3.41	0.587	2.82	1.46	1.08	0.82	0.26
11	Pre-operative	14.0	2.4	0.99	1.17	0.501	0.67	1.51	1.46	1.18	0.28
12	1st post-operative										
	2nd post-operative	10.6	2.1								
	3rd post-operative	9.7	2.2	0.74	0.59	0.080	0.51	0.67	0.45	0.23	0.22
	4th post-operative	9.1	2.4								
13	5th post-operative										
	6th post-operative	8.6	3.7	0.87	0.79	0.044	0.75	1.36	0.93	0.55	0.38
	7th post-operative										
	8th post-operative	8.3	3.1								
14	9th post-operative										
	10th post-operative	8.8	3.1	0.97	0.73	0.019	0.71	1.59	0.97	0.60	0.37
	11th post-operative										
	12th post-operative										
15	13th post-operative										
	14th post-operative	9.2	2.1	0.64	0.60	0.019	0.58	1.53	1.18	0.83	0.35
	15th post-operative										
	16th post-operative										
16	17th post-operative										
	18th post-operative			3.61	2.85	0.010	2.84	1.51	0.91	0.39	0.52
	19th post-operative										
	20th post-operative										
	58th post-operative	10.5	2.9								
	172nd post-operative	10.9	4.5								

The changes in the phosphorus and calcium metabolism which were encountered in Case 2, after removing the parathyroid tumor are summarized in table 7. The most striking change was the marked drop in the urinary calcium excretion. There was an immediate decrease in the calcium output due entirely to a fall in the urinary excretion. There was no evidence of any decided alteration in the calcium excretion in the stools. In 7 periods of low calcium intake before operation the average fecal excretion was 0.70 gram daily, while in four similar periods after operation the fecal excretion averaged 0.64 gram daily. In the preoperative period, 5, and the postoperative period 16, the high calcium intake was similar. In the first instance the average fecal excretion was 2.82 grams; in the second 2.84 grams daily. After operation there was a slight decrease in phosphorus output due to a slight decline in the urinary excretion.

NITROGEN, CREATINE AND CREATININE METABOLISM

A great part of the earlier literature on the function of the parathyroid glands concerns their relation to various aspects of nitrogen metabolism. Investigators have reported, in parathyroid tetany, an increased excretion of total nitrogen, ammonia, purine bodies, creatine, creatinine and guanidine bases. There seems to be no doubt of the increased nitrogen excretion in parathyroid tetany but Greenwald (38) showed this did not appear until after convulsions had started. Salvesen (64) found normal nitrogen excretion in latent tetany. After parathyroid extract in dogs, Greenwald and Gross (40) discovered an increased nitrogen excretion. Wilder's patient with hyperparathyroidism (79) stored considerable nitrogen during a ten-day period while on a high vitamin diet. The total nitrogen of Case 1 was studied before and after extirpation of a parathyroid tumor. For one hundred days before operation, while in the hyperparathyroid state, she appeared to be on nitrogen equilibrium. During this period the total nitrogen intake was 722 grams, the output 668 grams. For 100 days following the operation the nitrogen intake was 675 grams, the output 411 grams. At the end of this period, however, she was approximately in equilibrium again. These studies, therefore, do not indicate that there is any marked abnormality of nitrogen metabolism in hyperparathyroidism.

The relation of the guanidine bases to the parathyroid glands has aroused considerable interest, which has been stimulated by the fact that guanidine esters may produce a condition simulating parathyroid tetany. The large amount of creatine in muscle, the muscle phenomena associated with tetany and the chemical similarity of creatinine and the guanidine esters naturally suggests a possible relation between creatine metabolism and the parathyroids or calcium. Greenwald (38) and Burns (18) found a decrease in creatinine excretion after parathyroidectomy and Greenwald (38) an increase in creatine excretion. Hammett (43) reports that addition of parathyroid tissue to muscle extract retards the formation of creatinine which normally occurs. Woodman (82) found that feeding parathyroid to rats caused an alteration in the ratio of creatine and creatinine excretion resulting in the elimination of more creatine and less creatinine. Berglund, Medes and Lohmann (8) found no change in the creatinine in myasthenia gravis after increasing the serum calcium by parathormone.

Case 2 with typical hyperparathyroidism, yet free from complications and in good physical conditions except for a moderate anemia, offered ideal conditions for the study of creatine metabolism in this disease. The urinary excretion of creatine and creatinine nitrogen was determined during the eight days preceding and the eight days following operation. In the hyperparathyroid state an average of 25 mgm. of creatine nitrogen and 410 mgm. of creatinine nitrogen was excreted daily. Following the extirpation of a parathyroid tumor there was an immediate slight increase in creatine and a definite decrease in creatinine elimination. For the first four days a daily average of 81 mgm. of creatine nitrogen and 83 mgm. of creatinine nitrogen were excreted. Thereafter the excretion was similar to the preoperative days; creatine nitrogen 29 mgm. and creatinine nitrogen 406 mgm. daily. Except for the few days after operation the creatinine excretion was quite normal, corresponding to Shaffer's normal creatine coefficient.

SERUM ELECTROLYTES IN HYPERPARATHYROIDISM

That the activity of the parathyroid glands regulate the level of two inorganic constituents of the plasma and may cause profound alteration in their concentration appears of considerable physiological

interest. As far as we are aware no phenomenon in animal economy is entirely comparable to this unless we consider the remote similarity to the hormone influence on acid and alkali secretion of the stomach, pancreas and intestines. We have, at present, no knowledge as to the mechanism of this action. The characteristic alteration consists of a reciprocal rise and fall of the phosphorus and calcium; with a high calcium, a low phosphorus is usually encountered, and vice-versa, with high phosphorus as in tetany, low calcium is found. This behavior is typical of the effect of the solubility product of electrolytes in saturated inorganic solution and appears especially significant since the body fluids are in contact with an extensive surface of the undissolved phase, calcium phosphate in bone. But one mysterious feature appears. The quantity of calcium and phosphates in blood serum is far greater than can exist in simple aqueous solution of their salts. The parathyroids appear to increase the amount of calcium which may exist in serum yet in such a manner that it still maintains its reciprocal relationship to phosphorus as though following the laws of simple inorganic solutions. The marked physiological effects, which result from changes in serum calcium hint that the hormone may actually influence the ionic concentration instead of undissociated compounds as suggested by Greenwald and Gross (40).

The fact that the parathyroid glands may cause such marked changes in the calcium and phosphorus of the blood serum urged a careful investigation of the state of the other inorganic constituents in patients with hyperparathyroidism.

Collip (23) found that in dogs which had received parathyroid extract, carbon dioxide of serum gradually increased with a consistent slight increase in pH. The whole blood chloride was diminished but as the erythrocytes contain considerably less chloride than plasma this may have been due to the great concentration of blood. In a parathyroidectomized dog there was no change in the chloride of the blood after parathormone. Cantarow (19) et al. studied the effect on the blood of patients with pulmonary tuberculosis after relatively small doses of parathyroid extract. They found no constant change in plasma CO_2 or chloride. Brehme and György (14) found no change in the CO_2 in normal or tetanic children after parathormone although the alteration of phosphorus and calcium was quite typical. In 23

TABLE 8
Serum electrolytes of cases with hyperparathyroidism

Date	Calcium	Phosphorus	Carbon dioxide	Chloride	Protein	Total determined acids	Total base	Undetermined acids	pH	Remarks
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	
Case 1										
	<i>mgm. per 100 cc.</i>	<i>mgm. per 100 cc.</i>	<i>volumes per cent</i>	<i>mgm. per 100 cc.</i>	<i>per cent</i>	<i>mM</i>	<i>mM</i>	<i>mM</i>		
May 24, 1928.....	16.4	1.3	50.4	402	8.09	150.7	164.8	14.1		NaHCO ₃ , 8 grams daily for the previous 4 days
May 28, 1928.....	12.5	3.8	48.2		7.67					NaHCO ₃ , 8 grams and NaH ₂ PO ₄ , 5 grams daily for previous 4 days
June 1, 1928.....	12.8	5.8	46.0	399	6.85	147.0	161.4	14.4		NaHCO ₃ , 8 grams daily for previous four days
June 13, 1928.....	13.8	3.1	36.9		7.17					Blood lactic acid 18.9 mgm. per cent
June 23, 1928*.....	14.6	3.0	37.3	380	7.42	137.5	158.0	20.5	7.35	Irradiated ergosterol for the previous 8 days
July 3, 1928*.....	16.6	3.6	44.2	380	6.55				7.41	2 days after extirpation of parathyroid tumor
August 4, 1928*.....	12.9	3.0	41.3	358	6.34				7.37	9 days after extirpation of parathyroid tumor. Marked ketosis
August 11, 1928.....	7.4	11.3	35.3	382	6.67	140.9	160.3	19.4		35 days after extirpation of parathyroid tumor. No ketosis
September 6, 1928.....	9.1	2.5	51.3	413	4.91	142.7	158.3	15.5		
November 5, 1928.....	8.2	2.6		413						NH ₄ Cl 4.5 grams daily for 16 days

Case 2

	14.0	2.4	56.4	410	6.04	150.2	161.0	10.8	The day preceding operation 13 days after extirpation of parathyroid tumor
June 18, 1929.....				420	5.98	151.2	160.6	9.4	
July 1, 1929.....	9.2	3.1	48.5						

Case 3

	After low calcium diet				After high calcium intake for 8 days			
November 17, 1928.....	14.6	5.6	45.1	337	4.46	125.5	162.5	37.0
December 18, 1928.....	17.0	4.8	52.8	324	4.05	123.5	162.0	39.1

* During the absence of the authors, Dr. Alexis F. Hartmann of the Department of Pediatrics kindly made the studies.

patients with various diseases Csepai and St. Weiss (25) found no constant change in serum pH as the result of parathormone. Wilson and Riegel (81) studied the effect of parathormone on certain blood electrolytes in dogs. In the serum they observed a fall in the chloride and sodium and a delayed rise in potassium, while in the corpuscles, a decrease in the sodium and an increase in the potassium. There was little change in the water of the corpuscles or serum.

The total electrolyte equilibrium of the serum of three patients with hyperparathyroidism was studied in detail. The methods were exactly the same as those described in a previous publication (17). The results are recorded in table 8. The total determined acids in Column 6 show the sum of the bicarbonate, chloride, phosphate and protein, expressed in millimols of base combining capacity. The difference between the determined acids and the total base has been placed in Column 8, as undetermined acids, and includes the organic acid fraction and the normally small amount of sulfate.

As serum is practically neutral the total acids equal the total base and since there are no significant organic bases total base determinations may be used as a measure of that part of the total osmotic pressure which is due to the electrolytes. Gamble, Ross and Tisdall (35) and Peters, Bulger, Eisenman, and Lee (61) have emphasized the fact that the total base of serum is maintained at a remarkably constant level. They demonstrated how bicarbonate, chloride and protein may fluctuate to a greater extent yet reciprocate in their changes in such a manner as to keep the total acid relatively constant. The determinations of the total base on these patients will serve to emphasize further its remarkable stationary value. In the three patients it was continually quite normal and hardly varied outside the limits of experimental error even after considerable base had been administered in Case 1 and under conditions which had altered the serum anions. Case 1 reverted to severe tetany after removal of a parathyroid tumor and developed a ketosis without any change in the total base. Following extirpation of the parathyroid tumor in Case 2 there was no change in the total base concomitant with the striking decrease in serum calcium. There was no indication in the examinations of the other serum electrolytes of any abnormality characteristic of hyperparathyroidism except the shift in phosphate and calcium. It is

curious, however, that in both Case 1 and Case 2 the chloride concentration was definitely higher than that generally encountered in normal individuals. This was associated with relatively low bicarbonate in both patients; especially low in Case 1. In other words these patients had a mild chloride acidosis. But in neither case was this state altered by removing the hyperparathyroid condition.

The undetermined acid fraction in Case 2 was normal. The slight elevation of this fraction in Case 1 on August 11th, nine days after operation, was probably due to the organic acids resulting from the ketosis which developed on a low carbohydrate intake. No explanation can be offered for the slight increase on June 23rd. The two determinations of the serum electrolytes of Case 3 after a low calcium intake and after a high calcium intake both presented the same definite abnormality. The total electrolyte level as indicated by the total base was the same in both instances and normal. The undetermined acids were markedly increased while the other anions were low. This increase in undetermined acids was undoubtedly related to the associated kidney insufficiency and most likely represents a considerable retention of sulphate.

There appears to be no characteristic change in serum protein concentration associated with hyperparathyroidism. The first estimation of protein in Case 1 was definitely elevated. Thereafter the concentration tended to fall until the last determination almost four months later when it was quite low. The serum protein of the second case was moderately reduced. These downward deviations were possibly a result of chronic malnutrition. In neither case was the transition from hyperparathyroidism to hypoparathyroidism accompanied by any evident change in the concentration of serum protein. The very low protein of Case 3 was associated with extreme undernutrition.

In these studies we find, therefore, no evidence of any definite change in serum electrolytes which can be related to the increased activity of the parathyroid gland, except the alteration in phosphorus and calcium.

DISCUSSION

In 1923 Morton (60) suggested that in generalized osteitis fibrosa there might be an abnormality of calcium metabolism comparable to the abnormal carbohydrate metabolism of diabetes mellitus. He sug-

TABLE 9
*Calcium and phosphorus metabolism of Case 1. Four-day periods**

4-day periods	Period	Calcium				Phosphorus				Urine inorganic phosphorus	Urine titratable acidity cc. N acid per 100 cc.	Serum	
		Intake	Output	Urine	Stool	Intake	Output	Urine	Stool			Phosphate	Calcium
		grams	grams	grams	grams	grams	grams	grams	grams	grams		mgm. per 100 cc.	mgm. per 100 cc.
April 19 to 22.....	1	2.12	3.57	1.15	2.42	3.60	5.19	4.72	0.47	1.24			
April 23 to 26.....	2	2.13	4.01	1.21	2.80	3.60	4.38	4.04	0.34	1.27			
April 27 to 30.....	3	2.13	5.52	1.27	4.25	3.60	4.94	4.58	0.36	1.87			
May 4 to 7.....	4	2.35	5.47	0.97	4.50	3.60	6.03	5.79	0.24	2.53	4.32		
May 8 to 11.....	5	2.44	8.60	1.79	6.81	3.60	5.07	4.70	0.37	1.75	5.12		16.4
May 12 to 15.....	6	3.65	10.88	3.12	7.76	3.60	4.81	4.70	0.115	1.66	1.60		16.5
May 16 to 19.....	7	8.85	15.63	9.29	6.34	3.60	3.22	3.14	0.081	1.35	14.09	1.31	16.4
May 20 to 23.....	8	3.92	7.08	4.34	2.74	3.60	3.39	3.38	0.013	1.66	13.80		16.4
May 24 to 27.....	9	3.87	5.38	0.78	4.60	8.28	6.08	5.96	0.124	2.97	7.45	3.82	12.5
May 28 to 31.....	10	3.94	7.84	6.50	1.34	3.46	4.07	2.41	1.66	2.41	10.09	1.45	12.4
June 1 to 4.....	11	4.77	1.94	0.45	1.49	8.23	4.35	3.48	0.87	3.49	5.90	5.95	12.8
June 5 to 8.....	12	4.84	3.899	0.57	3.33	8.34	5.94	4.57	1.37	3.62	5.91		
June 9 to 12.....	13	4.74	5.89	0.43	5.46	8.39	7.45	4.71	2.74	4.18	8.18	3.0	13.8
June 13 to 16.....	14	2.69	2.69	0.75	1.94	3.82	7.84	2.64	5.20	2.15	4.77		
June 17 to 20.....	15	2.71	4.66	1.00	3.66	3.89	9.51	2.55	6.96	2.34	13.59	2.78	15.0
June 21 to 24.....	16	2.71	4.45	0.74	3.71	4.21	7.34	2.67	4.67	2.38	16.82	3.0	14.6
June 25 to 28.....	17	2.71	2.94	0.33	2.61	3.47	5.27	2.85	2.42	2.14	21.59		
June 29 to July 2.....	18	2.81	5.28	0.89	4.39	3.83	4.64	2.91	1.73	2.13	25.22	2.50	13.6
July 3 to 6.....	19	2.77	3.89	0.57	3.32	3.52	4.01	2.65	1.36	2.01	17.73	3.6	16.6
July 7 to 10.....	20	2.77	3.97	0.56	3.41	3.55	6.17	2.36	3.81	1.89	8.05	1.75	16.6
July 11 to 14.....	21	4.76	6.20	0.14	6.06	8.54	7.43	3.95	3.48	3.04	2.31		
July 15 to 18.....	22	4.82	10.40	0.16	10.24	8.40	7.38	4.08	3.30	3.89	5.38		

July 19 to 22.....	23	4.83	7.18	0.48	6.70	9.59	9.64	4.24	5.40	3.85	4.30		
July 23 to 26.....	24	4.83	11.50	1.00	10.50	9.57	10.90	4.37	6.53	4.17	9.68		
July 27 to 30.....	25	4.78	17.79	0.41	17.56	4.89	6.82	3.61	3.21	2.41	7.79		
July 31 to August 3.....	26	1.54	2.99	0.85	2.14	1.99	1.94	1.19	0.75	1.09	Alkaline	3.0	12.9
August 4 to 7.....	27	1.41	2.25	0.73	1.52	2.07	1.30	0.13	1.17	0.09	Alkaline	2.2	11.3
August 8 to 11.....	28	1.58	1.67	0.72	0.95	2.20	1.02	0.55	0.57	0.42	Alkaline	11.3	7.5
August 12 to 15.....	29	5.04	5.05	0.021	5.03	0.72	1.69	0.71	0.98	0.65	Neutral	3.0	7.5
August 16 to 19.....	30	6.70	1.13	0.018	1.11	1.93	1.11	0.59	0.52	0.52	Neutral	3.2	4.1
August 20 to 23.....	31	12.10	4.59	0.025	4.57	2.02	0.97	0.39	0.58	0.30	Neutral	3.0	5.0
August 24 to 27.....	32	8.95	7.78	0.105	7.67	2.27	1.78	0.190	0.59	0.105	Neutral	3.1	6.6
August 28 to 31.....	33	13.55	2.58	0.062	2.52	3.52	1.50	0.120	1.37	0.095	Neutral	2.3	7.8
September 1 to 4.....	34	15.52	4.23	0.139	4.09	3.51	0.61	0.090	0.52	0.082	Neutral	2.5	8.7
September 5 to 8.....	35	13.82	2.52	0.033	2.49	3.48	1.06	0.109	0.95	0.109	Alkaline	2.3	9.7
September 9 to 12.....	36	13.45	3.38	0.095	3.28	3.09	0.87	0.084	0.78	0.076	Alkaline	3.0	9.0
September 13 to 16.....	37	13.69	3.36	0.058	3.30	3.27	0.21	0.090	0.12	0.069	Alkaline	3.3	9.9

* The studies were continued during the following nine months and will be reported in detail in a paper on *hypoparathyroidism*

TABLE 9
Calcium and phosphorus metabolism of Case 1. Four-day periods*

4-day periods	Period	Calcium				Phosphorus				Urine inorganic phosphorus	Urine titratable acidity	Serum	
		Intake	Output	Urine	Stool	Intake	Output	Urine	Stool			Phosphate	Calcium
		grams	grams	grams	grams	grams	grams	grams	grams			mgm. per 100 cc.	mgm. per 100 cc.
April 19 to 22.....	1	2.12	3.57	1.15	2.42	3.60	5.19	4.72	0.47	1.24			
April 23 to 26.....	2	2.13	4.01	1.21	2.80	3.60	4.38	4.04	0.34	1.27			
April 27 to 30.....	3	2.13	5.52	1.27	4.25	3.60	4.94	4.58	0.36	1.87			
May 4 to 7.....	4	2.35	5.47	0.97	4.50	3.60	6.03	5.79	0.24	2.53	4.32		
May 8 to 11.....	5	2.44	8.60	1.79	6.81	3.60	5.07	4.70	0.37	1.75	5.12		16.4
May 12 to 15.....	6	3.65	10.88	3.12	7.76	3.60	4.81	4.70	0.115	1.66	1.60		16.5
May 16 to 19.....	7	8.85	15.63	9.29	6.34	3.60	3.22	3.14	0.081	1.35	14.09	1.31	16.4
May 20 to 23.....	8	3.92	7.08	4.34	2.74	3.60	3.39	3.38	0.013	1.66	13.80		16.4
May 24 to 27.....	9	3.87	5.38	0.78	4.60	8.28	6.08	5.96	0.124	2.97	7.45	3.82	12.5
May 28 to 31.....	10	3.94	7.84	6.50	1.34	3.46	4.07	2.41	1.66	2.41	10.09	1.45	12.4
June 1 to 4.....	11	4.77	1.94	0.45	1.49	8.23	4.35	3.48	0.87	3.49	5.90	5.95	12.8
June 5 to 8.....	12	4.84	3.899	0.57	3.33	8.34	5.94	4.57	1.37	3.62	5.91		
June 9 to 12.....	13	4.74	5.89	0.43	5.46	8.39	7.45	4.71	2.74	4.18	8.18	3.0	13.8
June 13 to 16.....	14	2.69	2.69	0.75	1.94	3.82	7.84	2.64	5.20	2.15	4.77		
June 17 to 20.....	15	2.71	4.66	1.00	3.66	3.89	9.51	2.55	6.96	2.34	13.59	2.78	15.0
June 21 to 24.....	16	2.71	4.45	0.74	3.71	4.21	7.34	2.67	4.67	2.38	16.82	3.0	14.6
June 25 to 28.....	17	2.71	2.94	0.33	2.61	3.47	5.27	2.85	2.42	2.14	21.59		
June 29 to July 2.....	18	2.81	5.28	0.89	4.39	3.83	4.64	2.91	1.73	2.13	25.22	2.50	13.6
July 3 to 6.....	19	2.77	3.89	0.57	3.32	3.52	4.01	2.65	1.36	2.01	17.73	3.6	16.6
July 7 to 10.....	20	2.77	3.97	0.56	3.41	3.55	6.17	2.36	3.81	1.89	8.05	1.75	16.6
.....	21	4.76	6.20	0.14	6.06	8.54	7.43	3.95	3.48	3.04	2.31		
.....	22	4.82	10.40	0.16	10.24	8.40	7.38	4.08	3.30	3.89	5.38		

gested that the calcium in the blood might be higher than normal and might occasion a loss of calcium through the kidneys. The accuracy of his analogy is well borne out by the studies of hyperparathyroidism. The general character of the abnormal metabolism of both diseases is similar. In diabetes a *deficient* production of an internal secretion results in an elevation of the blood sugar. In hyperparathyroidism an *increased* production of an internal secretion causes a rise of the serum calcium. In both conditions the alteration gives rise to certain general disturbances in the body and effects a loss of the involved substance through the kidneys.

Many of the clinical manifestations of hyperparathyroidism appear to be directly or indirectly related to hypercalcemia. From our knowledge of the influence of calcium on muscle physiology, symptoms referable to the muscular system could have been predicted. Muscle weakness, sometimes extreme, appears as a prominent feature. A decrease in muscle irritability was evidenced by three patients presented here who had absent or diminished response to faradic stimulation. It appears from numerous studies in the literature that an *increase* in the calcium concentration causes an *increase* in the tone of smooth muscle and of the heart (63) (16). The hypercalcemia of parathyroid extract appears to cause similar changes (32) (48). The cases of hyperparathyroidism reported by Gold (37) and by Boyd, Milgram and Stearns (13) had definite gastrointestinal symptoms which conceivably were due to the influence of high calcium on smooth muscle. Apparent circulatory failure developed in Case 3 as the serum calcium became very high after excessive calcium administration. It would seem that sudden cardiac episodes might be expected in hyperparathyroidism. Such an occurrence is suggested in the case of osteitis fibrosa reported by Dawson and Struthers (28) who succumbed four hours after unexpected and mysterious collapse. Two cases observed here became stuporous and irrational with exceedingly high serum calcium. The metastatic calcification is probably related to the hypercalcemia but we have as yet little knowledge concerning the mechanism of its production and its relationship to clinical features of the disease. As noted above it is most often found in the lungs, gastric mucosa and the kidneys, in tissues where acid is excreted. Cases in the literature with evidence of hyperparathyroidism have

TABLE 10

Calcium and phosphorus metabolism of Case 2. Four-day periods

4-day periods (1929)	Period	Calcium				Phosphorus				Serum phos- phate mgm. per 100 cc.	Serum cal- cium mgm. per 100 cc.
		Intake	Output	Urine	Stool	Intake	Output	Urine	Stool		
		grams	grams	grams	grams	grams	grams	grams	grams		
May 5 to 8.....	1	3.04	4.25	1.474	2.78	6.00	4.47	3.22	1.25	1.6	16.8
May 9 to 12.....	2	3.25	2.75	1.063	1.69	5.02	4.19	3.28	0.91		
May 13 to 16.....	3	4.18	5.19	2.220	2.97	6.23	5.85	4.78	1.07	1.8	15.2
May 17 to 20.....	4	3.79	5.13	2.162	2.97	5.52	5.39	4.32	1.07		
May 21 to 24.....	5	14.77	13.61	2.348	11.26	5.85	4.34	3.30	1.04		
May 25 to 28.....	6	15.34	19.69*	2.421	15.43*	5.84	10.45*	3.86	2.396*	1.9	14.9
May 29 to June 1..	7	3.30		1.835		4.83		4.19		2.1	14.0
June 2 to 5.....	8	3.68		1.978	Lost	5.36		4.69	Lost		
June 6 to 9.....	9	3.92	5.01	1.509	3.51	5.81	5.62	4.14	1.48		
June 10 to 13.....	10	4.00	4.97	1.900	3.07	6.11	5.35	3.71	1.64	2.6	14.1
June 14 to 17.....	11	3.96	4.69	2.004	2.69	6.04	5.84	4.71	1.13	2.4	14.0
June 18 to 21.....	12	2.98	2.39	0.320	2.07	2.69	1.82	0.92	0.90	2.1	9.7
June 22 to 25.....	13	3.58	3.19	0.176	3.02	5.44	3.75	2.21	1.54	2.4	9.1
June 26 to 29.....	14	3.89	2.91	0.075	2.84	6.36	3.92	2.42	1.50	3.1	8.3
June 30 to July 3..	15	2.58	2.41	0.078	2.33	6.13	4.70	3.31	1.39	2.1	9.2
July 4 to 7.....	16	14.46	11.40	0.041	11.36	6.06	3.67	1.59	2.08		

* The stool specimen in this instance was collected during two periods: 6 and 7, from May 25th to June 1st.

TABLE 11

Calcium and phosphorus metabolism of Case 3. Four-day periods

4-day periods	Period	Calcium				Phosphorus				Serum phos- phate mgm. per 100 cc.	Serum cal- cium mgm. per 100 cc.
		Intake	Output	Urine	Stool	Intake	Output	Urine	Stool		
		grams	grams	grams	grams	grams	grams	grams	grams		
November 9 to 12.....	1	2.17	4.34	1.107	3.23	3.82	2.76	1.63	1.13	2.6	15.4
November 13 to 16.....	2	2.57	7.00	1.101	5.90	3.57	3.36	1.68	1.68	3.57	14.6
November 17 to 20.....	3	1.72	4.35	1.011	3.34	2.62	2.82	1.35	1.47	5.6	14.6
November 21 to 24.....	4	2.53	2.71	0.724	1.98	2.94	2.44	1.47	0.97		
November 25 to 28.....	5	5.25	4.13	0.753	3.38	3.51	2.84	1.56	1.28		
November 29 to De- cember 2.....	6	5.14	3.42	0.561	2.85	2.95	2.28	1.27	1.01		
December 3 to 6.....	7	5.19	1.55	0.183	1.37	3.44	1.83	0.93	0.90	5.26	15.7
December 7 to 10.....	8	5.40	5.45	0.808	4.65	3.68	3.02	1.05	1.97		
December 11 to 14.....	9	14.26	10.83	0.760	10.07	3.84	3.05	0.68	2.37		
December 15 to 18.....	10	14.38	6.35	1.051	5.30	4.10	2.33	0.89	1.44	5.3	17.8
December 19 to 22.....	11	4.10	2.57	1.598	0.98	7.48	5.41	1.07	4.34	8.6	11.7

gested that the calcium in the blood might be higher than normal and might occasion a loss of calcium through the kidneys. The accuracy of his analogy is well borne out by the studies of hyperparathyroidism. The general character of the abnormal metabolism of both diseases is similar. In diabetes a *deficient* production of an internal secretion results in an elevation of the blood sugar. In hyperparathyroidism an *increased* production of an internal secretion causes a rise of the serum calcium. In both conditions the alteration gives rise to certain general disturbances in the body and effects a loss of the involved substance through the kidneys.

Many of the clinical manifestations of hyperparathyroidism appear to be directly or indirectly related to hypercalcemia. From our knowledge of the influence of calcium on muscle physiology, symptoms referable to the muscular system could have been predicted. Muscle weakness, sometimes extreme, appears as a prominent feature. A decrease in muscle irritability was evidenced by three patients presented here who had absent or diminished response to faradic stimulation. It appears from numerous studies in the literature that an *increase* in the calcium concentration causes an *increase* in the tone of smooth muscle and of the heart (63) (16). The hypercalcemia of parathyroid extract appears to cause similar changes (32) (48). The cases of hyperparathyroidism reported by Gold (37) and by Boyd, Milgram and Stearns (13) had definite gastrointestinal symptoms which conceivably were due to the influence of high calcium on smooth muscle. Apparent circulatory failure developed in Case 3 as the serum calcium became very high after excessive calcium administration. It would seem that sudden cardiac episodes might be expected in hyperparathyroidism. Such an occurrence is suggested in the case of osteitis fibrosa reported by Dawson and Struthers (28) who succumbed four hours after unexpected and mysterious collapse. Two cases observed here became stuporous and irrational with exceedingly high serum calcium. The metastatic calcification is probably related to the hypercalcemia but we have as yet little knowledge concerning the mechanism of its production and its relationship to clinical features of the disease. As noted above it is most often found in the lungs, gastric mucosa and the kidneys, in tissues where acid is excreted. Cases in the literature with evidence of hyperparathyroidism have

shown a high incidence of urinary tract disease. Nephrolithiasis with secondary pyelitis and cystitis has been common. The possible relationship of this phenomenon to the excessive urinary calcium excretion is obvious.

These studies have indicated that the metabolism of calcium and phosphorus in bone disease should be examined more critically. So often only the total calcium and phosphorus balance has been emphasized. Such factors as distribution of calcium and phosphorus between the urine and the feces appear to show at times certain characteristics, an emphasis of which may present important differential diagnostic features and therapeutic indications. In the literature there is great confusion even as to the terminology of bone diseases. One notes the tendency to use the term osteomalacia in connection with almost any generalized disease of the skeleton occurring in adult life. Even though etymologically the term could be used in a general sense, it seems desirable to limit it quite strictly. There seems to be ample evidence that there is a specific type of disease with a distinct pathological physiology for which this name should be reserved. Maxwell (57) points out that it must be differentiated from hyperparathyroidism. The calcium and phosphorus metabolism in the two diseases present striking contrasts. The study of Miles and Feng (59) has shown most completely the abnormalities encountered in osteomalacia. It demonstrates the low serum calcium, the diminished calcium output in the urine and the excessive excretion of both calcium and phosphorus in the stools. Earlier but less complete studies have shown similar changes. A case of non-puerperal osteomalacia investigated in this clinic has shown abnormalities identical with those presented by Miles and Feng.

It is apparent that the metabolic changes in hyperparathyroidism are quite unlike those of osteomalacia. Early in this report the chemical studies of Mandl (56), Gold (37), Hannon et al (44), Wilder (79), Boyd et al. (13) and Hunter (49) were briefly reviewed. They showed abnormalities which paralleled the changes recorded here, the most characteristic features being the hypercalcemia and hypercalciuria. Similar changes were found by certain earlier investigators who, however, did not determine serum calcium or examine the parathyroids. Again there is difficulty in the confused nomenclature of

these conditions to decide which of their cases should be included as hyperparathyroidism. The clinical features of some were so similar that there seems little doubt that they should be included in the group under discussion. One notes especially the case reported by McCrudden and Fales (55) as non-puerperal osteomalacia. This patient had multiple bone tumors, decalcification of the bones, difficulty in walking, gastrointestinal symptoms and kidney stones; a picture similar to Case 1 in this present report. Jacoby and Schrott (51) studied a typical case of osteitis fibrosa with the same characteristic changes in calcium metabolism. The case reported by Freund and Lockwood (34) as osteomalacia had less marked clinical characteristics but excessive urinary calcium excretion.

There are other studies showing alterations of the same character in multiple myeloma. Hypercalcemia in multiple myeloma has been reported by Charlton (20), Durman (31), Smith (72) and Soper and Stroud (74). Seegelman's (67) investigation of a case of multiple myeloma showed values for the calcium metabolism which were normal. Blatherwick (10) reports a case with a negative calcium balance and an increased output of calcium in the urine. Williams (80) and Currie (26) both record cases showing excessive urinary calcium excretion. The question arises whether these manifestations are the result of hyperparathyroidism or simply an incident to a disease causing in itself a rapid destruction of bone. From our knowledge of serum calcium regulation it seems unlikely that anything can cause such a constant high level of serum calcium except an increased activity of the parathyroid gland. It is difficult to cause more than slight changes in the serum calcium by giving large quantities of calcium by mouth. After intravenous administration the serum calcium promptly returns to normal. With the evidence at hand, it seems more logical to assume that hypercalcemia and hypercalciuria arising in multiple myeloma indicates that hyperparathyroidism has developed as a complication.

Suggesting this possibility that hyperparathyroidism may arise in generalized bone disease, it is well to point out that a diversity of experiments have shown the parathyroid to possess a remarkable power to develop hyperplasia. Significant appears the case reported by Klemperer (52) with metastatic bone carcinoma and a parathyroid

tumor, as well as the numerous cases of chronic nephritis with hyperplasia or tumor of the parathyroid gland.

While obvious treatment of hyperparathyroidism is the surgical removal of excessive parathyroid tissue, the therapeutic application of orthophosphate administration was suggested in a former section of this report. Caution should be employed in applying this procedure. One must be aware of the fact that phosphate may increase the intestinal excretion of calcium and thus exaggerate the negative calcium balance. There also appears the theoretical possibility of increasing the metastatic calcification. Orthophosphate administration may at least be reserved for treating alarming conditions which may arise with extreme hypercalcemia.

SUMMARY

A study of three cases of hyperparathyroidism, two with *osteitis fibrosa cystica* and the other with multiple myeloma, has presented certain abnormalities of calcium and phosphorus metabolism. Most characteristic of these were a high serum calcium, possibly accounting for many of the clinical features, and an excessive excretion of calcium by the kidneys, apparently explaining the urinary tract complications. The increase of calcium in the urine was sufficient to cause a negative calcium balance, although two patients showed, in addition, an excessive output in the stools. Low serum phosphorus was repeatedly found except in one patient with marked kidney insufficiency. Altered phosphorus metabolism was encountered but no specific abnormality presented. There was no indication that the total nitrogen metabolism or creatinine metabolism is abnormal in hyperparathyroidism. Although there were marked changes in calcium and phosphorus other serum electrolytes were normal.

By the administration of orthophosphate it was possible to cause an increase in the serum phosphate, a decrease in serum calcium, a reduction of the calcium in the urine to normal and establish a positive calcium balance. It appears, however, that a continuation of this procedure resulted in an excessive output of calcium in the stools.

Following parathyroidectomy in two patients the disturbances of calcium and phosphorus metabolism were reversed. The calcium almost disappeared from the urine and large amounts of calcium and

phosphorus were retained in the proportion in which they occur in the bone. One patient reverted to tetany which was almost fatal. It was controlled with difficulty by the administration of large amounts of parathormone and calcium.

The metabolism of hyperparathyroidism differs almost completely from that of osteomalacia. In the literature, however, great confusion exists in nomenclature. Cases have been called osteomalacia which probably were examples of hyperparathyroidism. This state is perhaps most often seen with von Recklinghausen's disease of bones, but accompanies hyperplasia of the parathyroid from a variety of causes which include multiple myeloma, extensive carcinoma of bone and possibly other destructive bone processes.

These studies indicate that improvement in the clinical state of hyperparathyroidism may be accomplished by the administration of orthophosphate. The possible dangers of such a procedure have been made evident. It would appear that the only effective method of treatment is the removal of parathyroid tissue.

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STUDIES IN CONGESTIVE HEART FAILURE

V. THE POTASSIUM CONTENT OF SKELETAL MUSCLE OBTAINED BY BIOPSY

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(Received for publication May 7, 1930)

As a result of their studies of the respiratory exchange of decompensated cardiac patients during and after exercise, Eppinger, Kisch and Schwarz (1927) concluded that the buffering power of the tissues was diminished. Laszlo (1928) found diminished phosphate content of the cardiac and skeletal muscle of such individuals. Harrison and Pilcher determined oxygen utilization during various degrees of edema (1930a) and oxygen debt in patients with congestive heart failure (1930b). In both studies the findings were believed to be indicative of diminished alkaline reserve in the muscles. Observations by Pilcher, Clarke and Harrison (1930) of the hydrogen ion concentration of the blood of patients with congestive heart failure after exercise lead to the same conclusion. In order to obtain more direct data on this point it was thought advisable to investigate the chemical changes in the tissues themselves. As potassium is the most abundant basic element in muscle tissue it seemed wise to study it first. Harrison, Pilcher and Ewing (1930) analyzed samples of cardiac and skeletal muscle obtained from patients who had died of congestive heart failure and found them to be abnormally poor in potassium. It seemed conceivable that such a phenomenon might be related to changes after death and consequently, before proceeding further with our studies on *postmortem* tissues, it was deemed wise to analyze tissues obtained during life. Such a procedure had the additional advantage of allowing us to make more than one observation on the same patient and hence to follow changes in potassium content under various conditions.

METHODS

Patients with various types of cardiac disease were chosen. All of them had or had had edema. Specimens of gastrocnemius muscle weighing one to two grams were removed under novocaine anesthesia with aseptic technique. Care was taken to infiltrate the anesthetic around, rather than in, the portion of muscle to be removed. The fresh muscle was weighed, dried to constant weight in an oven at one hundred to one hundred ten degrees Centigrade and then analyzed according to the technique used by Harrison, Pilcher and Ewing. Results obtained by this procedure have been found to be in fair agreement with those arrived at by a slower and somewhat more accurate method which will be published later.

Three patients were studied before and after the loss of edema. Single observations were made on one cardiac patient and on one individual with edema of renal origin. Investigations were carried out on five patients before and at intervals after the administration of potassium dibasic phosphate. This salt was given because Laszlo had found diminished phosphate and our analyses showed decreased potassium.

Edema was classified by the degree and extent of "pitting." An attempt was made to determine the length of time edema of the calves of the leg had been present but our figures in this regard represent only rough approximations.

RESULTS

Normal skeletal muscle contains about 25 to 28 per cent solids. The potassium content of dried muscle is approximately 1.2 to 1.5 per cent, and that of "wet" muscle is usually in the range of 0.26 to 0.38 per cent (Lematte, Boinot, and Kahane (1928); Norn (1929); Harrison, Pilcher and Ewing (1930)).

As can be seen from table 1, the percentage of solids was diminished in all of the subjects. The potassium content of the "wet" muscle was also invariably low. Dilution due to edema was one factor in this decrease. In some cases such as E. B. and H. C. a dilution with serum containing 8 per cent protein could explain the values found. In M. H. and T. R. the amounts of protein which would have had to

be present is impossibly high. Since, however, the edema fluid of cardiac failure contains a negligible amount of protein (Hass), it seems improbable that such dilution can account for the low values obtained. In subjects T. R. and M. H. loss of edema was followed by a rise in the potassium content of the dry muscle. E. G. and H. C. had, when edematous, normal amounts of potassium in their dry muscle. In this regard it may be of some significance that their edema was of relatively short duration, whereas, the other subjects who exhibited a

TABLE 1

The potassium content of the gastrocnemius muscle of patients with congestive heart failure

Subject	Etiological diagnosis	Date	Solids	Potassium in dry muscle	Potassium in wet muscle	Degree of edema	Duration of edema
		1929	per cent	per cent	per cent		
E. B.	Hypertension, auricular fibrillation	August 29	16.0	0.86	0.138	+	3 months
T. R.	Syphilis, aortic insufficiency, hypertension	August 26	20.2	1.03	0.207	+	2 months
		October 24	21.0	1.18	0.251	±	
M. H.	Arteriosclerosis, hypertension	September 21	13.8	0.88	0.121	+++	5 months
		October 2	18.3	1.60	0.295	0	
E. G.	Syphilis, aortic insufficiency	October 10	14.4	1.39	0.200	+++	2 weeks
		October 22	27.1	1.11	0.300	0	
H. C.	Nephrosis, no cardiac disease	July 31	16.3	1.21	0.201	++	3 weeks

diminished potassium content of the dried muscle had had edema for longer periods. E. G. had a higher potassium content of the dry muscle when edematous than when free of edema twelve days later.

These findings demonstrate that muscles as well as subcutaneous tissues become edematous in patients with congestive heart failure. They also suggest that the first effect of edema is that of simple dilution and that a later effect is actual loss of potassium from the muscle.

In table 2 are presented analyses on five patients before and after receiving potassium dibasic phosphate. In all of them the potassium

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TABLE 2

The potassium content of gastrocnemius muscle of patients with congestive heart failure before and after the administration of potassium dibasic phosphate

Subject	Etiological diagnosis	Date	Solids	Potassium in dry muscle	Potassium in wet muscle	Degree of edema	Remarks
			per cent	per cent	per cent		
T. P.	Syphilis, aortic insufficiency, angina pectoris	1929 June 4	19.4	0.69	0.135	+	Before receiving K_2HPO_4
		July 3	18.2	0.92	0.167	0	After receiving K_2HPO_4 6 grams daily for four weeks
G. H.	Hypertension	July 10	20.2	1.06	0.213	±	Before receiving K_2HPO_4
		July 28	19.5	1.48	0.288	±	After receiving K_2HPO_4 14 grams daily for two weeks
F. J.	Asthma, chronic bronchitis	June 18	19.8	0.96	0.190	+	Before receiving K_2HPO_4
		July 31	18.0	1.28	0.231	+	After receiving 6 grams daily for 5 weeks
		October 14	21.6	1.20	0.260	±	After receiving K_2HPO_4 6 grams daily for fifteen weeks
J. A.	Hypertension, arteriosclerosis, auricular fibrillation	August 23	16.6	0.85	0.142	+++	Before receiving K_2HPO_4
		September 23	21.5	1.11	0.240	0	After receiving K_2HPO_4 6 grams daily for four weeks
H. M.	Rheumatic heart disease, mitral stenosis, aortic insufficiency	October 24	23.8	1.16	0.274	0	Before receiving K_2HPO_4
		December 6	19.9	1.73	0.345	±	After receiving K_2HPO_4 6 grams daily for six weeks

content of the wet and dry muscle was greater after the salt had been administered. One of the subjects (J. A.) had marked edema at the time the first piece of muscle was taken and no edema when the second specimen was removed. T. P. had slight pitting at the time of the control analysis and no edema later. In their cases the rise in the potassium content might conceivably have been due to loss of edema. However, such an explanation does not hold in the other three cases for they had no less edema at the time of the second analysis.

In only one of the five cases (J. A.) was the water content of the muscles much greater before potassium was given than after it had been administered. Consequently, loss of edema could not have been the cause of the rise in potassium content observed. Although it is conceivable that the changes observed might have occurred spontaneously, it seems more likely that they were related to the administration of the salt.

Another point of interest can be observed in both tables 1 and 2. Even though there was no clinical sign of edema, i.e., no pitting, subjects who had once had edema usually continued to have abnormally low total solid content in their *gastrocnemii*. Four of the five subjects studied had distinctly low muscle solids when entirely free of pitting.

SUMMARY AND CONCLUSIONS

1. The water content of pieces of gastrocnemius muscle removed by biopsy from patients with cardiac edema was invariably increased. The percentage of solids was correspondingly decreased. These changes usually persisted after clinical signs of edema had disappeared.

2. The potassium content of the wet muscle from edematous patients was invariably abnormally low. The amount of potassium in the dry muscle was usually but not always diminished. As edema decreased the potassium content of the wet muscle rose in three subjects; that of the dry muscle increased in two of them.

3. The administration of potassium dibasic phosphate was followed by a rise in the potassium content of the muscle.

4. The findings reported in the previous paper of this series, were not due to postmortem changes.

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THE ACTION OF HISTAMINE ON THE PANCREAS

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(Received for publication May 5, 1930)

Claude Bernard (1856) and Heidenheim (1883) in their general physiological studies, initiated the earliest ideas of pancreatic function, but it remained for the classical experimental work of Pawlow (1901) and Bayliss and Starling (1902) to demonstrate the fundamental concepts of the physiology of the pancreas as a gland of external secretion. Pawlow observed that a flow of pancreatic juice occurred after the entry of the chyme into the duodenum. He suggested that this effect was due entirely to reflex vagal activity and supported this idea by obtaining a secretion on directly stimulating the vagi. That this was not the whole truth was shown by the experimental studies of Bayliss and Starling (1) in which it was indicated that pancreatic secretion could also be initiated by the contact of the duodenal mucosa with a weak acid solution, or by the injection of an extract of the mucosa itself; this whole phenomenon being due to the elaboration and circulation of a chemical stimulant of hormonal qualities. After extensive experiments these workers established the specificity of this hormonal stimulation, and noted the absence of any action on the salivary and gastric glands. This humoral theory was amply supported by Farrell and Ivy (2) who obtained active and copious secretions in a denervated transplanted portion of the pancreas after the ingestion of a meal. This transplant also yielded a small continuous resting secretion containing the digestive enzymes.

That the two methods of pancreatic stimulation induce secretions differing in composition is now well known,—“vagal” juice being small in volume but rich in protein and enzymes,—while “secretin” juice is copious in quantity, poor in proteins and enzymes but definitely alkaline due to the carbonate content. Atropine paralyzes the vagal

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stimulation but does not affect the secretin response. Pilocarpine on the other hand causes an elevation in the enzymes secreted. Babkin (3) had shown that the zymogen granules in the pancreatic acinar cells greatly diminish after vagal stimulation. Under normal conditions, therefore, there appears to be a dual and yet coördinated activity of the two types of pancreatic stimuli. In the presence of achlorhydria, the action of secretin is probably negligible and vagal juice plays a dominant rôle in pancreatic digestion. The production of a copious flow of dilute bicarbonate solution may not be essential to normal digestion, although its main function would appear to ensure, in the presence of an acid chyme, an optimal reaction in the chyle for the maximal activity of the pancreatic digestive enzymes. A number of our investigations suggest that the achylia of pernicious anemia in no way impedes the secretion, or the activity of the pancreatic ferments. It is likely that this secretion is due to vagal stimulation and that the hormonal activity in complete achylia is slight or absent since the secretion is practically abolished by atropine. It is noteworthy that the flow of dilute bicarbonate solution which is probably the response to hormonal activity, is not present in achylia where alkalization of the gastric contents is unnecessary. It is interesting that enzyme production and hydrogen ion concentration, the regulation of which is so important for intestinal digestion, should be separately initiated.

HISTAMINE

Histamine, when injected subcutaneously, stimulates the secretion of gastric juice. Cushny (4) remarks that this substance also stimulates pancreatic secretion, although a careful search through the literature has failed to produce any experimental data to corroborate this statement. It was this note in Cushny's book which gave the writers a desire to investigate the action of histamine on the pancreas. It was thought possible that a method of studying pancreatic function might be established if it could be definitely demonstrated that histamine stimulates the pancreas as it does the gastric glands. With this object an experimental method was devised to determine whether histamine, on subcutaneous injection, would increase the volume of pancreatic secretion or the concentration of enzyme therein.

All the experiments have been carried out in normal healthy children ranging in age from six weeks to twelve years. Children were used for the experimental work because it was also desired to obtain collateral data on the enzyme and volumetric secretion at various ages during childhood. Again, for many reasons, the most important being the considerable length of the experiments in some instances, children were found to be more suitable than adults. A few observations on adults have been made.

METHOD

A quiet and cooperative patient was essential; the child lying down in bed inclined toward the right side. An interval of about six hours was allowed after the last meal; an early breakfast was given, and the experiment commenced in the forenoon. A special small torpedo shaped aspirating tip, capable of passing through the nose of the smallest infant, and yet heavy enough to be influenced in the stomach by gravity, was devised. This will be described in a clinical note. The tip, at the end of a fairly stiff rubber tubing, was introduced into the duodenum, and its position verified by fluoroscopic examination. It was left in place throughout the experiment; so long as the child remained fairly quiet no displacement occurred. Constant drainage of the duodenal contents was maintained, and the amount of secretion measured every ten or fifteen minutes. Obviously, uninterrupted supervision of the child was required throughout the experiment to aspirate and collect the fluid, and to eliminate all extrinsic inhibitory or disturbing factors.

The passage of gastric juice into the duodenum could be readily detected by the change in appearance of the aspirate. All fractional samples of fluid obtained were verified by determination of the pH—colorimetrically after dialysis, according to the method described by Marriott and Davidson (7).

In order to determine the gastric secretory response to the same dose of histamine, a separate experiment was carried out on the day following the pancreatic experiment. One of us (A. V. N.) has already reported upon the dosage of histamine ("Imido-Roche"), for children of different age groups, which causes a maximal flow of acid gastric juice (6).

The concentration of enzymes in the duodenal fluid was determined by the methods of McClure, Wetmore and Reynolds (5).

RESULTS

After preliminary experiments, a standard procedure was developed. Some thirty subjects were investigated with very satisfactory and uniform results. Only a few of these experiments are shown in the charts but they are typical examples of the findings in all.

On charts A and B the gastric and duodenal response to an injection of histamine in two typical cases are presented. They show that within a few minutes (15 to 45 minutes) after the injection, the gastric secretion is stimulated whereas the duodenal flow is simultaneously diminished. The gastric and duodenal secretions are apparently inversely affected. As the histamine effect disappears the gastric secretion falls and the duodenal secretion tends to return to its previous resting level. The enzyme content in the pancreatic secretion also shows a corresponding lack of stimulation or even a fall. These findings were typical of those obtained in all the experiments.

Charts C, D and E depict the same findings as charts A and B; but in addition there is shown the stimulation of the pancreas after the histamine action has ceased, by the entrance of gastric secretion into the duodenum. The upper line on the graphs shows the pH of the fluid collected at the corresponding times in the duodenum. In all instances, the reaction obtained confirmed our interpretation as to the nature of the fluid collected. It will be noticed that there is a slight alkaline "tide" in the duodenum coincident with the phase of histamine activity during which there is a large outpouring of acid gastric secretion into the stomach.

It is interesting to note that following the entrance of the acid gastric contents into the duodenum, the pancreatic flow which follows is profuse, watery, and alkaline, ("secretin juice"). In chart C this is shown very well, the secretion reaching a rate of 240 cubic centimeters per hour. In one case it rose to 300 cubic centimeters per hour. After cessation of the acid stimulation, the rate of secretion again falls to the resting level of the individual concerned.

Table 1 shows the rate of secretion into the duodenum and its reaction during the pre- and post-histamine phases. In each case the rate

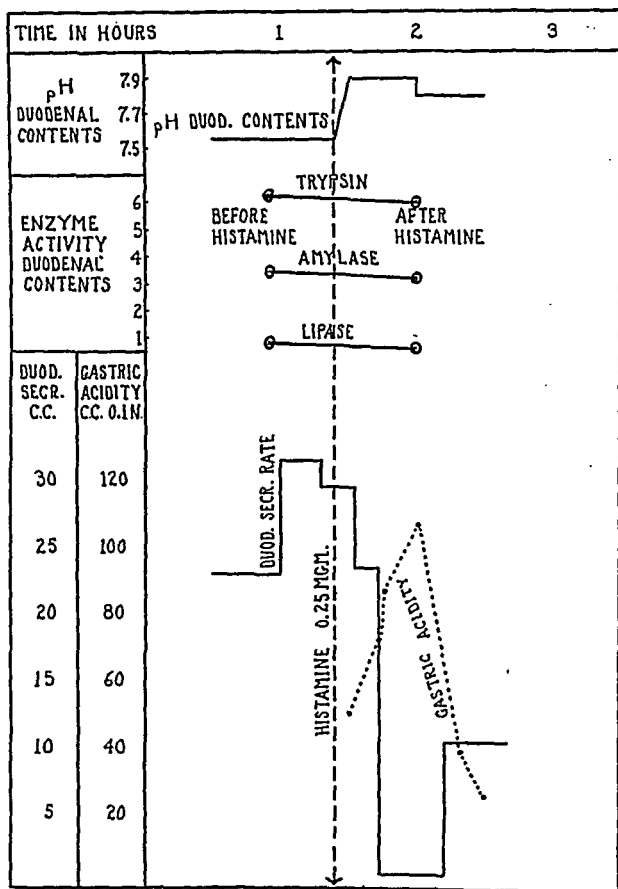


CHART A. GASTRIC AND DUODENAL RESPONSE TO HISTAMINE. R. N. 9 YEARS
6 MONTHS

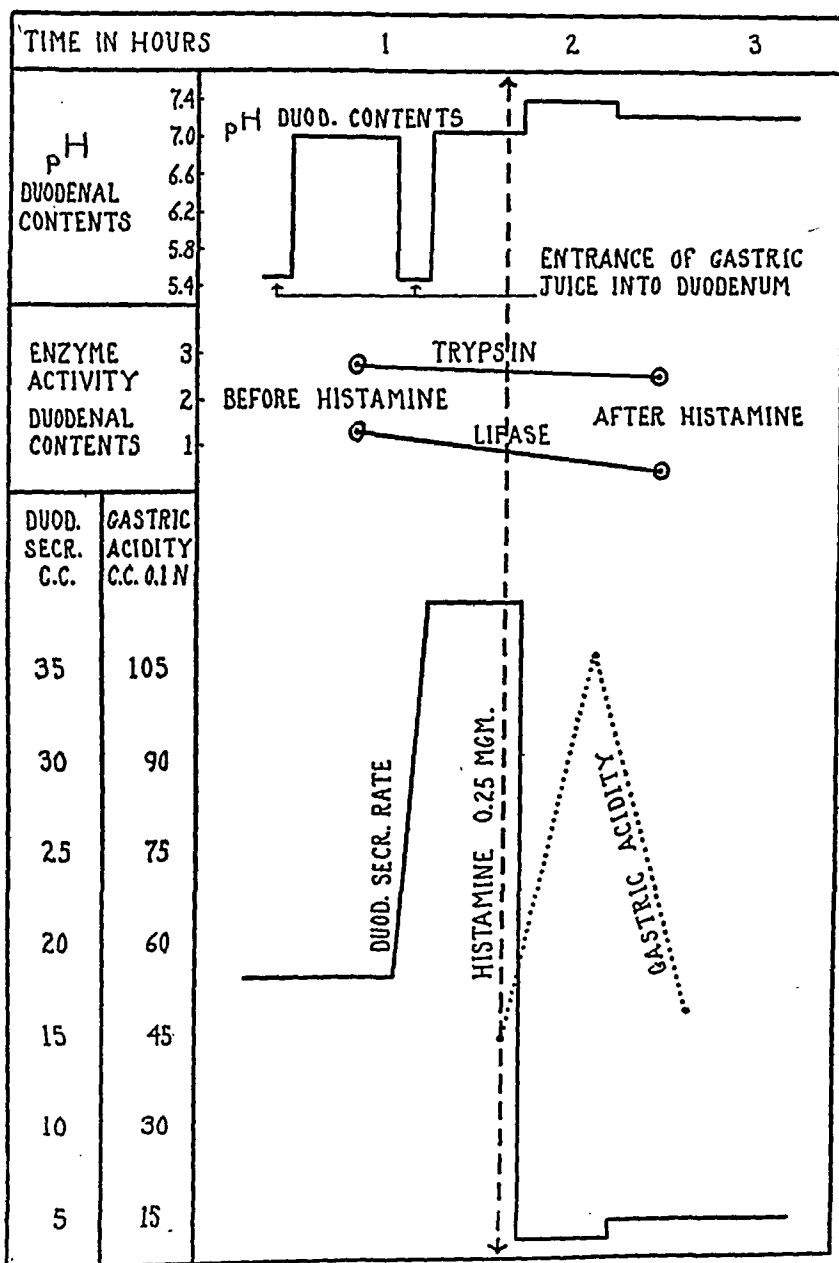


CHART B. GASTRIC AND DUODENAL RESPONSE TO HISTAMINE. E. S. 9 YEARS, 11 MONTHS

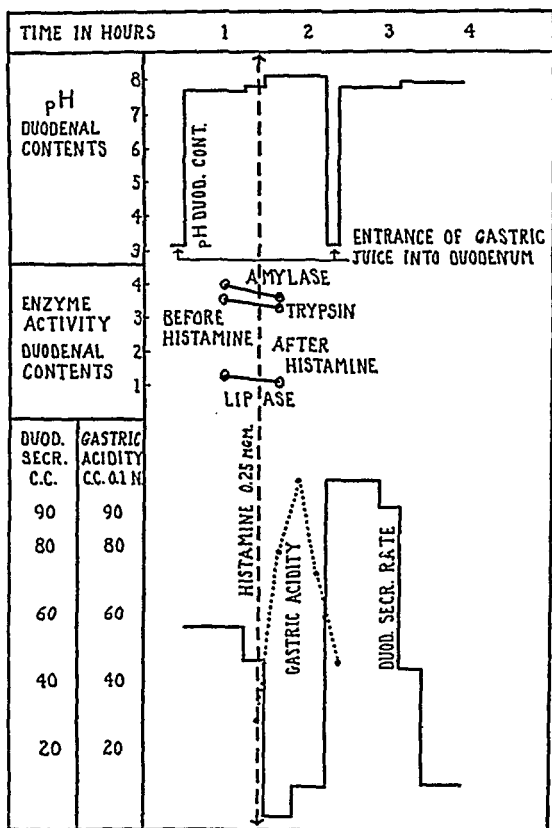


CHART C. GASTRIC AND DUODENAL RESPONSE TO HISTAMINE. J. C. 7.6/12 YEARS

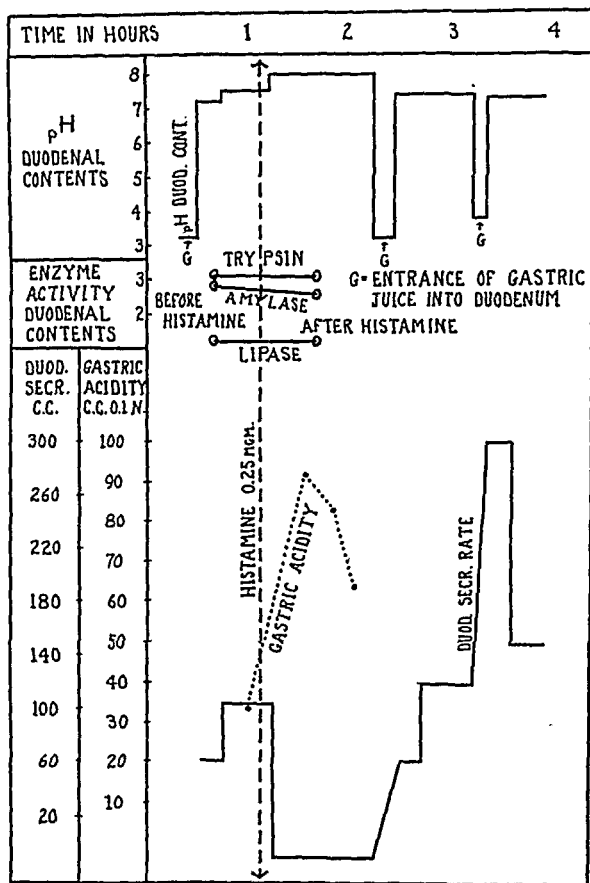


CHART E. GASTRIC AND DUODENAL RESPONSE TO HISTAMINE. A. S. 11 YEARS

2. Histamine caused no increase of either the volume of pancreatic secretion or of its enzyme activity.

3. An increase in the alkalinity of the duodenal contents (alkaline tide) accompanied the secretion of acid into the stomach in response to histamine.

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A COMPARISON OF THE UREA NITROGEN CONTENT OF CUTANEOUS AND VENOUS BLOOD BY MICRO GASOMETRIC ANALYSIS

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(Received for publication April 8, 1930)

Svensgaard (1) using the micro urea method of Rehberg (2) compared the urea concentrations in blood from the arm vein with that of cutaneous blood from the lobe of the ear. In 29 of 32 analyses of the blood of normal fasting subjects she found the urea of the capillary blood higher than that of the venous blood. The average difference was 10 per cent of the venous urea content, but individual differences were as high as 24 per cent. Such results, if confirmed, would indicate that the use of cutaneous blood from the ear for analysis could introduce errors up to 24 per cent in tests of renal function, such as the blood urea clearance used in this clinic (3, 4, 5), which depend upon blood urea determinations.

It was therefore thought advisable to make a series of determinations of the urea content of samples of cutaneous and venous blood taken simultaneously and both analyzed by the Van Slyke micro gasometric method (6) in order to ascertain whether the variations found by Svensgaard could be confirmed. A report of the analyses is given in this paper.

METHODS

Into each of two small tubes (10 mm. inner diameter and 50 mm. in length) a little powdered heparin was introduced. The tubes were then rotated horizontally until the heparin was evenly distributed, and were then inverted so that only the finely powdered heparin which clung to the walls of the tube remained. After preliminary rubbing to produce hyperemia, the lobe of the ear was punctured deeply enough to insure a free flow with only slight pressure, and sufficient blood was collected in one tube. From time to time during the collection the puncture wound was wiped clean so that only fresh blood would be obtained. Vena puncture was done immediately afterward, either with or without stasis. The samples were taken immediately to the laboratory and 0.2 cc. portions measured

TABLE 1

Comparison of the urea nitrogen content of cutaneous and venous blood

Subject	Venous blood urea nitrogen			Cutaneous blood urea nitrogen			Absolute difference venous minus cutaneous	Difference as per cent of venous	Remarks
	Individual analyses	Mean	Maximum deviation	Individual analyses	Mean	Maximum deviation			
	mgm. per 100 cc.	mgm. per 100 cc.	per cent	mgm. per 100 cc.	mgm. per 100 cc.	per cent	mgm. per cent		
W. N.	9.2 9.5 *	9.4	2.1	9.5 9.6 9.7	9.6	1.0	-0.2	2.1	Normal
A. H.	9.6 10.0	9.8	2.0	10.1 9.8	10.0	2.0	-0.2	2.0	Normal
R. H.	10.2 10.0 *	10.1	1.0	10.0 10.1 10.2	10.1	1.0	-0	0	Normal
W. K.	11.5 10.5 10.7	10.9	6.0	11.3 12.0 11.9	11.7	3.4	-0.8	7.3	Normal
M. L.	13.8 13.8 13.8	13.8	0	14.1 13.6 14.1	13.9	2.2	-0.1	0.7	Normal
A. S. A.	14.8 15.1 14.9	14.9	1.3	15.0 14.7 14.6	14.8	1.4	+0.1	0.7	Normal
A. S. A.	17.1 17.4 17.7	17.4	1.7	16.8 17.6 17.3	17.2	2.3	+0.2	1.2	Normal
A. C.	9.6 9.4 9.6	9.5	1.1	9.3 9.7 9.7	9.6	3.1	-0.1	1.1	Nephritic, during diuresis
J. D.	40.8 41.3 40.8	41.0	0.8	40.4 39.8 38.9	39.7	2.0	+1.3	3.2	Nephritic, urea therapy

Average of all venous bloods 42.4 mgm. per cent. Average of all cutaneous bloods 42.4 mgm. per cent.

* Discarded because of gross error.

TABLE 1—*Concluded*

Subject	Venous blood urea nitrogen			Cutaneous blood urea nitrogen			Absolute difference venous minus cutaneous	Difference as per cent of venous	Remarks
	Individual analyses	Mean	Maximum deviation	Individual analyses	Mean	Maximum deviation			
	<i>mgm. per 100 cc.</i>	<i>mgm. per 100 cc.</i>	<i>per cent</i>	<i>mgm. per 100 cc.</i>	<i>mgm. per 100 cc.</i>	<i>per cent</i>	<i>mgm. per cent</i>		
J. E.	{ 63.6 64.3 63.7	63.9	0.6	{ 64.0 63.7 63.8	63.8	0.3	+0.1	0.2	Nephritic, urea therapy
M. G.	{ 101.1 102.1 102.2	101.8	0.7	{ 103.9 103.6 *	103.8	0.2	-2.0	2.0	Nephritic. Uremia
D. McK.	{ 117.9 118.7 *	118.3	0.3	{ 116.6 117.3 117.8	117.2	0.5	+1.1	0.9	Nephritic. Uremia
J. O'M.	{ 129.9 129.9 130.6	130.1	0.4	{ 130.1 131.0 129.2	130.1	0.7	0	0	Nephritic. Uremia

into tubes containing 1 cc. of 0.02 N lactic acid as described by Van Slyke. As analyses were made in triplicate, three accurately calibrated pipettes were used. After taking the three samples of blood from one source they were washed and thoroughly dried before using again. After addition to lactic acid, the samples stood at room temperature. As each analysis takes 15 minutes, and as at least two blank determinations were made with each experiment, the last sample stood approximately 2 hours before analysis, but it seemed to make no difference.

The method of collecting blood from the ear into a tube rather than by drawing directly into a pipette was chosen because it is easier, and measurement of the 0.2 cc. samples can be made more comfortably and hence with greater accuracy in the laboratory. There is no trouble from clotting.

Bleedings were done between one and two hours after lunch, except in three experiments, which were done about the same length of time after breakfast. No attempt was made to control diet.

RESULTS

Inspection of table 1 shows that the gasometric method, using 0.2 cc. of whole blood, gives results of sufficient accuracy to demonstrate

any essential difference in the concentration of urea nitrogen between cutaneous and venous blood. Occasionally, through faulty technic, one gets a reading that is obviously incorrect and has to be discarded. This happened but four times in this series of 72 analyses. In the case of subject W. K., it is likely that one of the stopcocks was leaking slightly, which led to the introduction of an error much larger than usual. For this reason the experiment should probably be discarded but it is included here for the sake of completeness. No experiments have been omitted from the table.

Excluding the observation in question, five of the remaining twelve sets of analyses showed a higher concentration in cutaneous blood (average 1.6 per cent), five showed higher concentration in venous blood (average 1.2 per cent), and two showed no difference. The average concentration in the twelve samples of both venous and cutaneous blood is 45.0 mgm. per cent urea nitrogen. The maximum difference in any one experiment was only 3.2 per cent.

DISCUSSION

Marshall and Davis (7) in a study of the distribution of urea in the body concluded that "the urea content of all organs and tissues is approximately uniform, and approximately equal to that of blood, both in normal conditions, and when there is an abnormally large amount of urea present." Furthermore "when urea in solution is injected intravenously, it diffuses to all parts of the body almost instantly, the diffusion being complete in a few minutes." Gad-Andresen (8) came to essentially the same conclusions with regard to equilibrium between tissues and blood, and in addition found that the distribution coefficient between body secretions and blood plasma was unity, with the exception of sweat and tears, in which the concentration of urea was higher than in the plasma. He found, however, that plasma contained more urea than the erythrocytes, the distribution coefficient being between 0.72 and 0.82. Both Marshall and Davis and Gad-Andresen found that fat is an exception to the general rule in that it is relatively poor in urea. The experiments of Bollman, Mann, and Magath (9) seem definitely to have established that urea formation is a function of the liver only. If equilibrium exists between blood and tissues, one would expect to find no difference in the concentration of urea between venous and cutaneous blood.

So far, no explanations have been found to account for the apparent differences noted by Svensgaard. The fact that the mean error of her tests was ± 1 mgm. urea (0.47 mgm. urea N) might have contributed some, but the average difference noted was much greater than this. Returning again to her observations on normal subjects, the concentration of urea in capillary blood varied between 4 and 22 per cent higher than in venous blood. After administration of urea, or of protein the differences were from 1 to 7 per cent. In seven cases of icterus the variation was from 2 to 23 per cent, in five cases of nephritis from 1 to 13 per cent, and in 8 of 9 diabetics from 2.2 to 24 per cent.

SUMMARY AND CONCLUSIONS

1. The concentration of urea nitrogen in the blood from ear puncture has been compared with that from the vein in 13 experiments on 6 normal and 6 nephritic subjects, without regard to food intake.
2. No difference between venous and cutaneous blood exceeding the limit of experimental error was found to exist.

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FURTHER OBSERVATIONS ON EXPERIMENTAL AORTIC INSUFFICIENCY

II. CINEMATOGRAPHIC STUDIES OF CHANGES IN VENTRICULAR SIZE AND IN LEFT VENTRICULAR DISCHARGE

BY CARL J. WIGGERS, HAROLD THEISEN AND HARLEY A. WILLIAMS

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(Received for publication April 30, 1930)

INTRODUCTION

In 1923, Wiggers (1) ventured the declaration that existing experimental evidence contradicted the view so prevalent among clinicians, viz., that a large proportion of the blood ejected during systole flows back through a leaking aortic valve during diastole. Since that time the question has been re-investigated by other methods both in animals and in man. In a more recent review (2) this work also was critically considered and reasons were given for believing that crucial proof has still not been adduced in favor of the view that the regurgitating volume in aortic insufficiency is very considerable. The radiographic and fluoroscopic researches on heart size in the dog after production of experimental lesions were among the work reviewed. Bazett and Sands made several reports (3, 4 and 5) of their investigations on surviving animals. They found that rupture of an aortic cusp caused an immediate reduction in the area of the x-ray shadow; but they attributed this to a coincident cardiac acceleration. Obviously, no conclusions as to the magnitude of reflux could be drawn from such observations. They found, however, that the heart size gradually returned to normal with the lapse of time, while the cardiac acceleration persisted. This, together with the postmortem findings that the left ventricles had hypertrophied and their cavities had increased in size, inclined these investigators to the belief that considerable regurgitation must have occurred. To us the validity of such deductions appears somewhat questionable. But granting this, the obvious corollary would also appear to follow, viz., that the increase in diastolic size due to

3. The size of the ventricular outline can be registered at successive short intervals during systole and diastole rather than during maximum diastolic relaxation only, as in ordinary x-ray exposures.

METHODS

The heart of a dog, anesthetized with sodium barbital, was exposed and supported in a cradle of pericardium made by stitching its cut

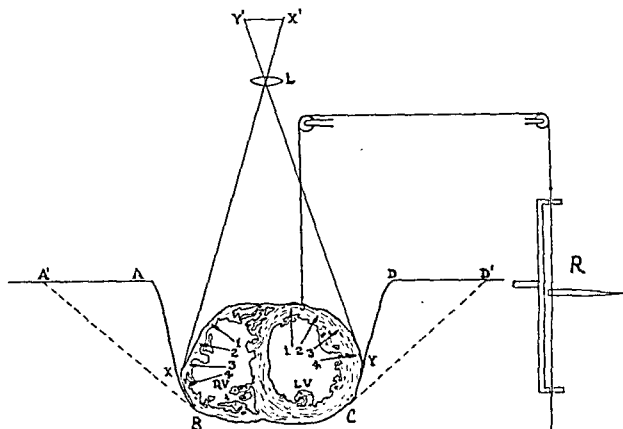


FIG. 1. SCHEMATIC DIAGRAM ILLUSTRATING SUSPENSION OF HEART, REGISTRATION OF ANTEROPOSTERIOR DIAMETERS AND CINEMATOPHOTOGRAPHY

A, B, C, D and *A', B', C', D'*, two methods of heart suspension by pericardium; *R, V* and *L, V*, right and left ventricles respectively; *x-y*, plane of frontal projection; *L*, camera lens; *x'-y'*, image on film; *R*, needle recording changes in anteroposterior diameter of left ventricle. Further discussion in text.

edges to the chest wall. Artificial respiration was kept low and regular, so as to prevent the plane of the heart from moving forward more than 3 mm. during inflation. This was actually controlled by registering the anteroposterior movement of the left ventricle on a drum by means of a vertically moving stylus. The principle of this simple expedient is illustrated in figure 1 and actual records of the movements are shown in figure 2.

regurgitation could not have been greater than a similar increase occasioned by a change in rate from 133 to 70 beats per minute. According to experimental work on normal hearts this is approximately 10 per cent. Herrmann (6), who made extensive studies upon surviving dogs, also found that rupture of the aortic cusps causes no immediate increase in the areas of x-ray shadows; on the contrary, the area actually decreased in some animals. Enlarged heart shadows were found only in the later stages when hypertrophy was actually present. It is obviously impossible to separate the effects of regurgitation from those due to muscular hypertrophy; Herrmann regarded the latter as the essential cause. Eyster (7), however, obtained earlier evidence of enlargement in similar experiments; the size of the x-ray shadows increased for 3 or 6 days and then gradually returned to normal after another period of about four days.

No attempts have been made to explain these contradictory results, but several reasons suggest themselves to us:

1. The cardiac acceleration may have been of lesser degree in the experiments performed by Eyster.
2. Differences in the method of rendering valves insufficient (e.g., rupture vs. incision) may be accountable for the supervention or absence of secondary circulatory changes.
3. Changes in diastolic volume of the dog's heart may not always be detectable by changes in the outline or area of x-ray shadows, owing to predominant anteroposterior enlargement. Eyster (7) particularly points out that the apex of the left ventricle is most easily stretched.
4. Considerable regurgitation may conceivably occur at the expense of auricular inflow without great additional distention of the ventricle. Experiments recently reported by Wiggers and Green (8) indicate that this mechanism of accommodation obtains under the most favorable conditions that can be created in a perfused heart.

Some of the difficulties encountered in determining and evaluating the changes in cardiac outline by means of x-ray shadows from intact animals can be obviated by recording the changes in size of the exposed heart by the cinematographic method. The following advantages are obvious:

1. The rate of the heart can be controlled artificially, thus eliminating the possible influence of heart rate changes on ventricular size.
2. The outlines of the ventricles can be accurately determined.

3. The size of the ventricular outline can be registered at successive short intervals during systole and diastole rather than during maximum diastolic relaxation only, as in ordinary x-ray exposures.

METHODS

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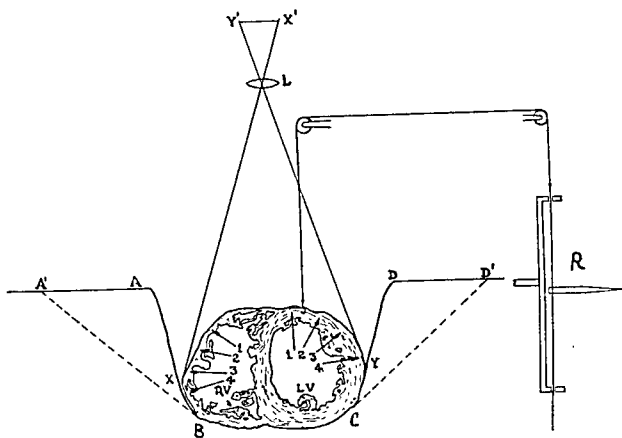


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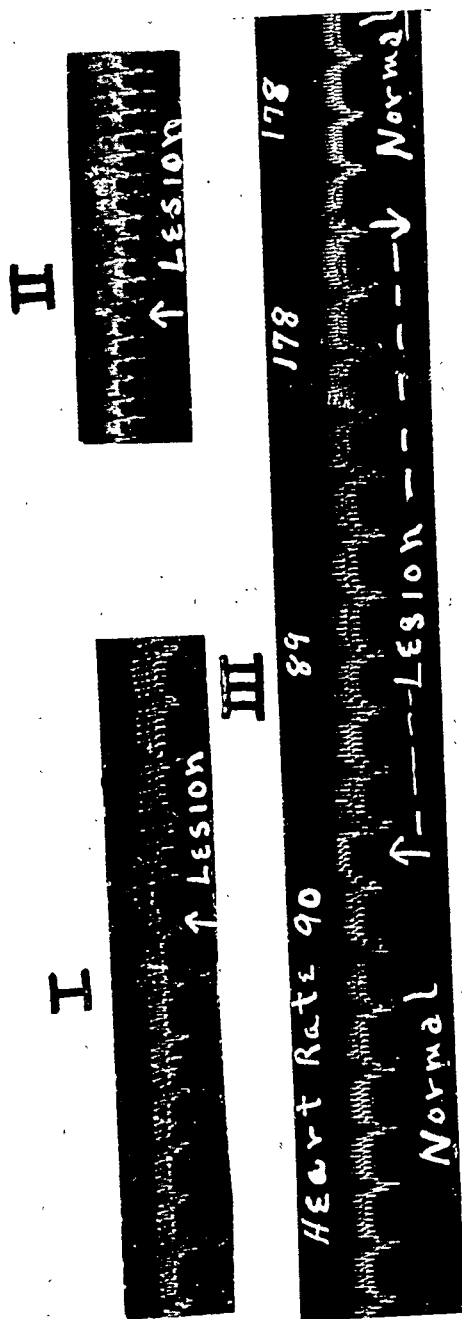


FIG. 2. THREE RECORDS SHOWING EFFECT OF AORTIC INSUFFICIENCY ON ANTEROPOSTERIOR MOVEMENTS OF LEFT VENTRICLE

I, curve showing irregular changes for several beats after a lesion, followed by resumption of normal movements (experiment C-486, II); II, curve showing lesion produced without mechanical disturbance of position (experiment C-472, I); III, curve showing effect of lesion at natural nodal rate of 90 and at artificial rates of 178 (experiment C-487, III, a-d). Further discussion in text.

In these, as in the first experiments on aortic insufficiency reported by one of us (9), an aortic leak was produced by a metal sound with a plunger, the instrument being introduced through the ventricular musculature of the left ventricle and entered into the root of the aorta. When the plunger was withdrawn, communication between the open end of the sound in the aorta and the two side openings of the tube lying within the ventricle was established. The diameter of the tubes varied from 5.5 to 9.3 mm. and created an insufficiency ranging from one-half to seven-eighths the size of the aortic orifice. As a rule areas equal to one-half to three-fourths that of the aortic orifice were employed because they produced no evidence of stenosis when the plunger was in place.

As previously intimated, the rate of the heart was kept constant. To accomplish this and yet maintain a sufficiently slow rate, the S-A node was first clamped in some animals. In a comparatively large percentage of cases, this caused a slow A-V nodal rhythm. By applying somewhat faster rhythmic break shocks to the right auricle, a comparatively slow and constant artificial tempo was maintained.

Synchronous moving pictures and optical aortic pressure tracings were taken before, during and at various intervals after creation of an insufficiency. In other experiments, we studied the changes after discontinuing a lesion by pushing the plunger home.

The method of recording arterial pressure curves has been described in detail by one of us (10). The principle of the photographic arrangements for obtaining moving pictures is obvious from the diagram of figure 1. If a moving picture camera with a suitable lens (L) is placed at a proper distance from the heart, a small picture ($x'y'$) of the plane $x-y$ is photographed on the film.

Moving pictures were taken on 16 mm. panchromatic film by means of a Bell-Howell camera equipped with a 2.5 cm. F-2.7 Zeiss lens. Sufficient illumination was secured from three 500 watt Mazda lamps equipped with reflectors to permit the use of a diaphragm stop between 2.7 and 4. The moving picture camera was rated to have a speed of 32 exposures per second. Actual tests showed that after the first 3 or 4 pictures and until the fully wound camera had run for 15 seconds the interval of exposure was exactly 0.02 second and the interval of shutter closure was 0.011 second. Individual pictures were thus taken every

0.031 second. The camera was placed at a distance of one foot from the anterior surface of the heart and photographed a picture which approximately filled each frame of the exposed film. The animal board was slightly tilted so that the surface of the heart was parallel with the surface of the film in the camera. After each observation, numbers or data written with chalk on a small black plate were photographed, thus enabling identification of films. Correlation of these pictures with optical pressure pulses was obtained by recording the occasional closing and opening of two signal magnets in the same circuit.

After development and conversion into a positive film, each frame was projected as a stationary picture, the surface area of which was six times that of the actual outline. The successively projected areas were drawn on a roll of semitransparent paper lying flat against a glass pane and so arranged that it could be pulled down from a roll. Space was saved by allowing an overlapping of the drawings of successive heart areas. Subsequently, each area was measured by means of a planimeter and the values expressed in cm^2 were plotted on coordinate paper on which the abscissae represented intervals of 0.031 second (cf. figs. 3, 4 and 5).

The physical sources of error introduced by movements of the heart, by changes in respiration, and by the "personal factor" in redrawing and measuring were carefully studied by Strughold (11) in this laboratory and will be reported elsewhere in detail. His studies justified the conclusion that the maximal total error may reach 10 per cent but, by attention to experimental details, this can be greatly reduced. Furthermore, much smaller errors in individual measurements are easily detected by plotting the successive values of surface area measurements for two or more consecutive beats. This may be illustrated by means of the curves of figure 3. A line connecting the successive dots usually gives curves having the general form of volume curves. If an occasional dot falls above or below such a line, as in the case of those labeled X, an error is involved. Since we are primarily concerned with the minimal and maximal measurements and their difference, such aberrant values may be disregarded. To guard against the introduction of similar errors in the case of the largest and smallest areas in any cycle, it is important to measure at least two

successive beats. The curves to be acceptable must not show a variation greater than 5 per cent.

After adopting all these technical precautions and also those foreseen by Strughold, we found to our regret that still another source of error remained. Strughold had happily avoided this pitfall by making his chest openings rather large; we, on the contrary, fell into it in some of our experiments by making—as we believed—a slight improvement in the operative technic. We refer to the manner in which the heart is suspended in the pericardium. If, as shown in the cross-section diagram of figure 1, the ventricles rest upon the pericardium stitched to a widely opened thorax, ample room for lateral expansion exists. This is illustrated by the lines $A^1 B C D^1$ of figure 1. If, however, the chest opening is reduced to $A D$ and the sides of the pericardium $A B$ and $C D$ consequently form a more acute angle with the ventricles, an increase in diastolic ventricular size due to any cause will distribute itself in the directions indicated by arrows 1, 2 and 3, but not in the direction indicated by the arrow 4. Under these circumstances, enlargement of the heart occurs in an anteroposterior direction, but no variation in the lateral diameter $x-y$ and, consequently, in the photographed diameter x^1-y^1 can occur.

The error is more significant when, as in our experiments on aortic insufficiency, the increase in the frontal projection of the two ventricles is effected through an increase in the size of the left ventricle alone. Consequently, we stress the surgical detail essential to successful application of the method; the thoracic opening must be made large and the angle between pericardial and ventricular surfaces must be kept great enough to permit free lateral expansion of the ventricles. The selection of round-chested dogs for experiments of this sort is of great help in accomplishing these aims.

Detailed analysis of representative results

A comprehensive consideration of our results may advantageously be preceded by a detailed discussion of several representative experiments:

The three graphs of figure 3 are introduced partly to show the character of our negative results, partly to demonstrate the effects produced by lung inflation.

Graph A shows the changes in cardiac size previous to an aortic leak. The differences in diastolic size are such as are common when lung inflation comes into play. The first beat was recorded at the end of deflation, the second showing a somewhat greater diastolic size, during beginning of inspiration. They emphasize the importance of selecting beats during comparable respiratory phases, preferably those during the phase of lung deflation.

Graph B represents the 9th and 10th beats after production of an insufficiency. The corresponding changes in the aortic pressure curves reproduced as segments A and B in figure 6 demonstrate clearly that an effective insufficiency had been created.

Graph C represents the outline curves obtained after insufficiency had been maintained for 4 minutes. The corresponding optical pressure curves of segment C in figure 6 show definitely that the vigor of ventricular ejection must have increased.

In spite of the circumstantial evidence of an augmented systolic discharge presented by the pressure pulses (segments B, C, fig. 6),—viz., the larger pulse pressure, coupled with a higher systolic pressure—neither the plots of figure 3 nor actual figures of more extensive measurements give any direct evidence that either the systolic discharge or the diastolic size had increased. Such observations accord with the equally negative results of investigators who have studied the effects of aortic insufficiency in intact animals by x-ray studies. In view of our previous discussion we are not inclined, however, to accept such demonstrations as evidence that the systolic discharge remained unaltered and that no regurgitation occurred. Indeed, graphic curves showing the changes in anteroposterior diameter supply direct evidence to the contrary. As illustrated in figure 2, I, such records unfailingly show an increase in amplitude of the excursions and an elevation of the base line indicating that the diastolic anteroposterior diameter increased and the amplitude of contraction became larger. We must therefore conclude that the changes in cardiac volume mirrored themselves entirely as variations in the anteroposterior diameter of the ventricle. Reference to our experimental notes verifies the suspicion that lateral expansion was prevented by the restricting action of the pericardial support.

The second series of outline curves selected for discussion and repro-

duced in figure 4 were obtained from an animal in which particular care was taken to permit adequate lateral expansion. The chest was widely opened and the pericardial surface made a large acute angle with the surface of the heart. The natural heart rate after clamping the S-A node was 90 per minute. The normal outline curve of the ventricles

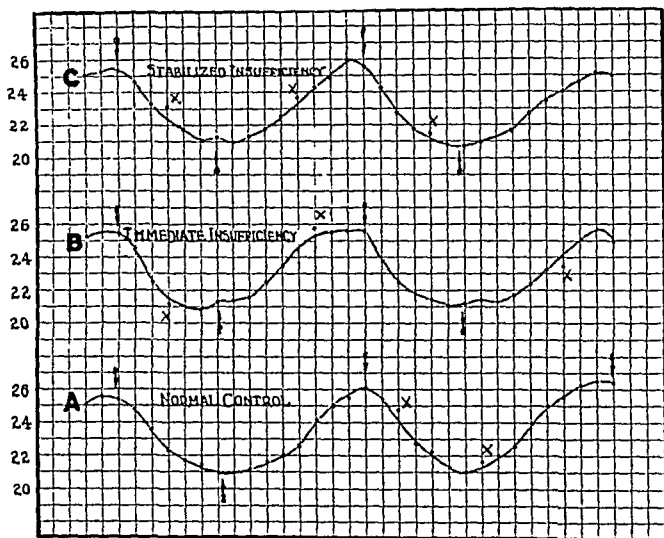


FIG. 3. THREE SETS OF CARDIAC OUTLINE CURVES DURING TWO SUCCESSIVE SYSTOLES AND DIASTOLES

Ordinate figures $\times 10 =$ sq. cm. of projected areas; abscissae = 0.031 second; letters A, B, C indicate correspondence with curves of figure 6; arrows indicate beginnings and ends of systole. Discussion in text. (Experiment C-486, II, a-b.)

(D) shows all the characteristics of a normal volume curve, including the long period of diastasis. From moving pictures taken shortly after the production of a leak, the outline curve (E) was plotted. The heart rate remained approximately the same. Curve E shows both an increase in diastolic size and a larger difference between maxi-

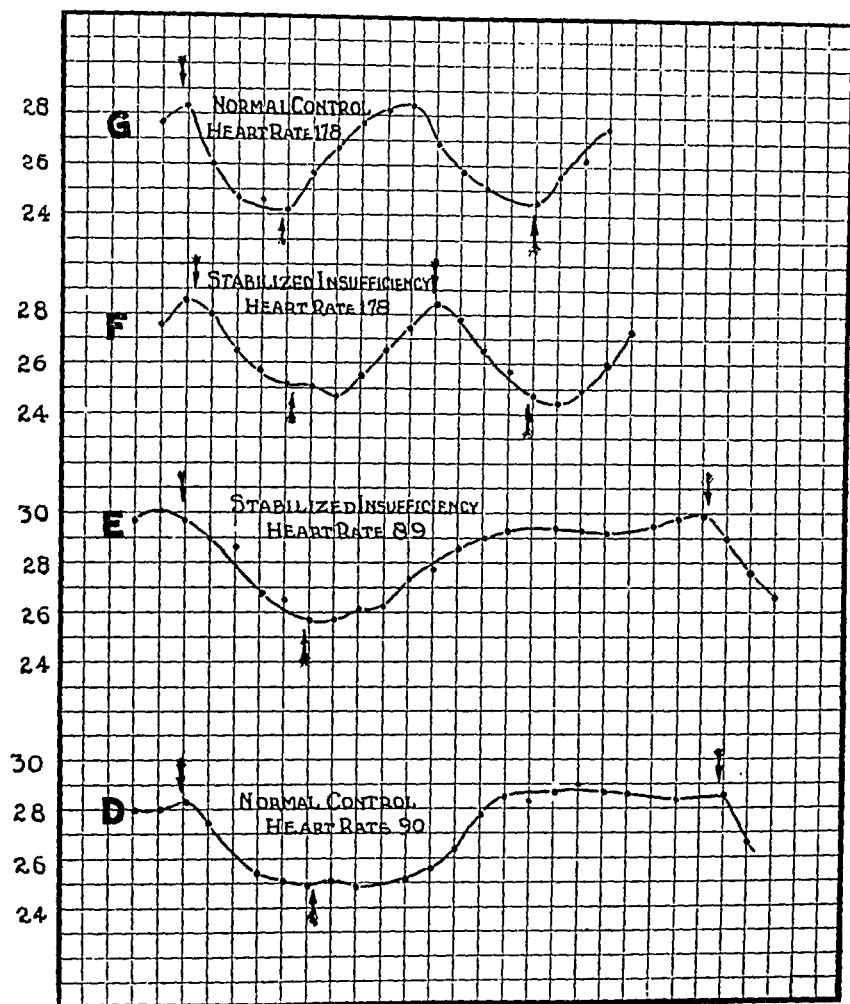


FIG. 4. FOUR GRAPHS SHOWING HEART SIZE CHANGES DURING SYSTOLE AND DIASTOLE WITH NORMAL AND INSUFFICIENT AORTIC VALVES AND AT DIFFERENT HEART RATES

Abscissal and ordinate values as in figure 3; arrows designate beginnings and ends of systole; letters *D*, *E*, *F*, *G* indicate correspondence with curves of figure 6. Discussion in text. (Experiment C- 487, III, a-d.)

mal and minimal size during the heart cycle. The optical pressure curves corresponding to these graphs are reproduced as segments *D*

and E of figure 6 and the records of anteroposterior changes are reproduced in figure 2, III.

Such curves leave no doubt of the fact that changes in ventricular volume after an aortic insufficiency are correctly reproduced by cinematographic registration when ample room for lateral expansion is provided. The limitations of such a method of support are, however, exemplified by a consideration of the two other graphs of figure 4. Desiring to study the influence of heart rate and duration of diastole on the magnitude of regurgitation, we artificially increased the heart rate to 178, while a constant size of leak was maintained. Finally, while the rapid rate continued, normal valvular conditions were restored by thrusting back the plunger. The changes in the pressure pulses are shown in segments F and G of figure 6 and the alterations in anteroposterior diameters in segment III of figure 2. Graph F in figure 4 shows the effects of increasing the heart rate. We note a decrease in diastolic volume and a moderate diminution in systolic discharge. The contour of the plotted curves are not particularly smooth, however, and the successive beats vary considerably as to minimal sizes. The upper curve showing a curve of successive outlines after restoration of normal valvular action is more regular, but neither the maximal nor minimal sizes differ appreciably from those of the curve below.

This lack of difference occurred in spite of ample room for lateral changes in size, and in spite of the fact that records of anteroposterior diameters (fig. 2, III) showed significant variations. Observations showed, however, that the experimental conditions were unfavorable for accurate filming of the cardiac outline. When the size of the ventricles decreased visibly in consequence of so great an acceleration, the pericardial space available was far too large; the ventricles in consequence flopped about in such a way that changes in focus probably resulted. Under such conditions an accurate comparison of changes in ventricular size is impossible. We enter so fully into these technical details in order to emphasize the reserve and caution that must be exercised in evaluating results.

As a final set of records for detailed analysis, we present in figure 5 the most pronounced effects on ventricular size and output we have succeeded in obtaining. They show in succession (II) a normal con-

trol curve, (*I*) a curve during an aortic insufficiency, (*J*) a normal curve after abrogation of the lesion and (*K*) a curve during a second aortic insufficiency. The corresponding optical pressure curves are shown as segments *H*, *I*, *J* and *K* of figure 6. Curves such as these give evidence that creation of a large aortic insufficiency can increase both the diastolic size and the extent of the systolic contraction.

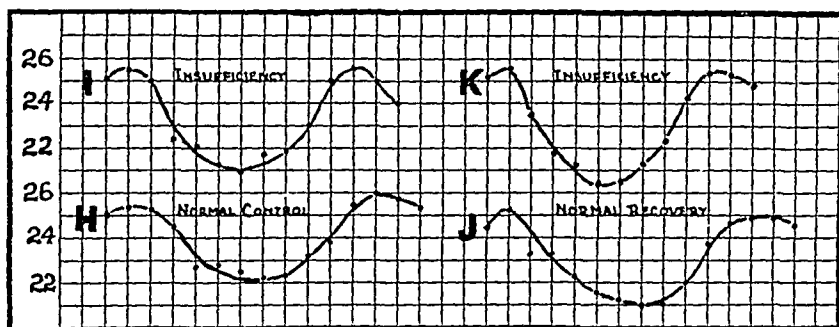


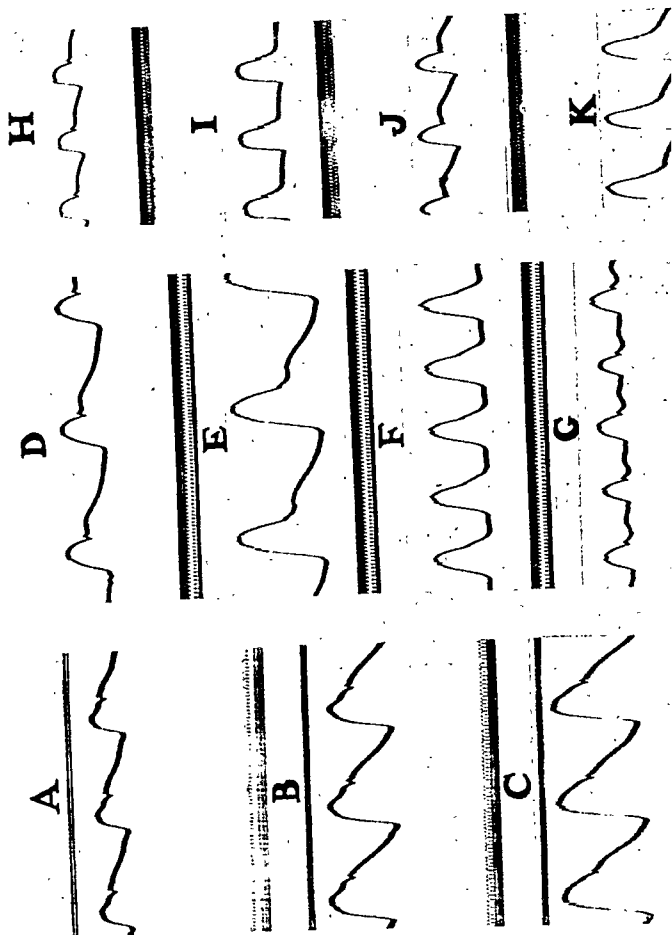
FIG. 5. FOUR GRAPHS SHOWING HEART SIZE CHANGES DURING SYSTOLE AND DIASTOLE WITH NORMAL AND INSUFFICIENT VALVES

Letters *H*, *I*, *J*, *K* indicate correspondence with curves of figure 6. Abscissae and ordinates as in figure 3. Discussion in text. (Experiment C-472, I, a, b, c and II, a.)

The differences in the curves do not, however, permit us to draw direct conclusions as to the magnitudes of the systolic strokes and, by inference, of the degree of regurgitation. In the first place, the increase in size probably occurs entirely in the left ventricle, whereas the curves represent areas in which the right and left ventricles normally have ratios ranging from 10:9 to 10:8. Furthermore, the curves represent changes in the frontal projection of the heart outline rather than true volume curves, regardless of certain resemblances in form. It is not improbable however that the relationships between volume

FIG. 6. THREE SERIES OF OPTICAL PRESSURE PULSES FROM AORTA SHOWING DYNAMIC CHANGES PRODUCED BY AORTIC LESIONS

Letters of segments correspond to those on graphs of figures 3, 4 and 5. Distances from base line give relative changes in systolic and diastolic pressure changes correctly. Time 0.02 second. Discussion in text.



and projected areas established by Bardeen (12) and Skavlem (13) apply also to our measurements. We have attempted, therefore, by use of Skavlem's formula ($S.D. = 0.44 \times A^{3/2}$) to translate the difference between systolic and diastolic areas directly into terms of systolic discharge. Doing this, in the case of the first two curves of figure 5, we find that the differences between the largest and smallest areas amount to 34 cm.² and 44 cm.² respectively. After reducing these values by one-sixth, to allow for magnification in projection, and applying Skavlem's formula, we find that the stroke volume of the two ventricles was 5.9 cc. before the lesion and 8.7 cc. after the lesion, giving a net increase of 2.8 cc. Applying a similar procedure in the case of the second set of curves, we calculate the stroke volume to be 8.2 cc. previous to the lesion and 9.6 during a lesion; an increase of 1.4 cc. Since, however, the increases in stroke volumes so determined are entirely due to left-sided effects, these values should be added to the normal stroke volumes of the left ventricle alone, i.e., roughly, to half the calculated stroke volume for the two ventricles.

The percentage increase in left ventricular discharge can then be calculated from these values by taking the left ventricular discharge during an insufficiency as a base. In the case of the experiment being analyzed the following steps become obvious:

	Curves H-I cc.	Curves J-K cc.
Stroke volume of two ventricles (normal).....	5.9	8.2
Probable stroke volume of left ventricle (normal).....	3.0	4.1
Increase in stroke volume of left ventricle (insufficiency)	2.8	1.4
Total stroke volume of left ventricle (insufficiency)...	5.8	5.5
Percentile increase in left ventricular discharge.	48.0	25.0

Comprehensive analysis of results

Having delineated the value and limitations of the method and analyzed the probable and definite information to be derived from the results of individual experiments, we are in a position to consider the results of all experiments in a more comprehensive fashion.

Complete calculations and analyses of changes in the outline curves following aortic insufficiency were made in 23 experiments, selected from a much larger number carried out on 10 different dogs. The heart rate was maintained constant in each set of observations, but

in different experiments ranged from 84 to 170 per minute. In 7 instances the diastolic size was not altered and the systolic discharge either remained unaltered or decreased slightly during existence of an aortic insufficiency.

Four of these negative experiments are satisfactorily explained by improper pericardial suspension; in each, the anteroposterior diameter increased during diastole. The remaining three, in which the minimal systolic area actually diminished during a lesion cannot be definitely accounted for.

The data from the remaining 16 experiments in which the systolic discharge increased are shown in table 1. In most instances, the increase was chiefly or solely due to the increase in maximum diastolic size; minimal systolic size changing but slightly. Experiments 468, V, c and 486 II, however, form two exceptions in which diastolic size diminished and the greater systolic discharge is clearly attributable to a predominant decrease in systolic size. For this reason these results are also questioned. This leaves 14 positive experiments which warrant further deduction. In these, the increases calculated by taking the systolic discharge during insufficiency as a base range from 16 to 31 per cent and from 41 to 58 per cent in two groups of 7 experiments each, the average being 36.4 per cent.

The factors determining the magnitude of the increase could not be satisfactorily analyzed. The increase seemed to bear no definite relation to heart rate, nor to the size of the leak. The heart rates in the two groups listed above were 170-90, 170-84 respectively, i.e., all degrees of increases were found at all heart rates examined. In experiment 478 the percentile increase appeared to be greater at slower rates, but in experiment 472 the reverse seemed to be the case.

DISCUSSION

Several investigators have carefully studied by means of x-ray methods the effects produced by acute aortic lesions on the maximal diastolic size of the ventricles. Our results indicate that the method is not destined to cast much light on the question as to the magnitude of the regurgitation. Even in our most favorable experiments, the increase in area was comparatively small when the heart rate was kept constant, recognizable differences being evident only when the cardiac outlines were magnified at least 6 times.

TABLE 1

Data from 16 experiments showing increased systolic discharge

Experiment	Stage	Heart rate	Maximum diastolic size $\times 6$	Minimum systolic size $\times 6$	Difference in areas $\times 6$	Calculated systolic discharge of two ventricles	Probable systolic discharge of left ventricle	Percentage increase systolic discharge. Left ventricle
		<i>per minute</i>	<i>cm.²</i>	<i>cm.²</i>	<i>cm.²</i>	<i>cc.</i>		
468	IIa	162	271	236	35	6.2	3.1	
	b	162	283	235	48	10.0	6.9	+55
468	IVa	162	262	222	40	7.6	3.8	
	b	162	272	228	44	8.7	4.9	+22
468	Va	170	277	247	30	4.9	3.0	
	b	170	293	260	33	5.7	3.8	+21
	c		262	224	38	7.0	4.9	+39
469	IIa	152	204	177	27	4.2	2.1	
	IIIa		214	181	33	5.7	3.6	+41
472	Ia	162	255	221	34	5.9	3.0	
	b		257	213	44	8.7	5.8	+48
	c	125	252	210	42	8.2	4.1	
	IIa		254	207	47	9.6	5.5	+25
	b		249	208	41	7.9	4.0	
	c		249	208	41	7.9	4.0	
474	I	150	284	220	64	15.2	10.7	+58
		150	275	230	45	9.0	4.5	
	II	160	271	221	50	10.6	5.3	
			282	229	53	11.6	6.3	+19
			272	222	50	10.6	5.3	
478	I	96	338	64	74	19.0	9.5	
			350	267	83	22.6	13.1	+27
			335	261	74	19.0	9.5	
	II	100	336	260	76	19.8	9.9	
			349	262	87	24.3	14.4	+31
			336	256	80	21.4	10.7	
	V	88	361	295	66	16.0	8—	
			368	285	83	22.6	14.6	+45

TABLE 1—*Concluded*

Experiment	Stage	Heart rate	Maximum diastolic size $\times 6$	Minimum systolic size $\times 6$	Difference in areas $\times 6$	Calculated systolic discharge of two ventricles	Probable systolic discharge of left ventricle	Percentage increase systolic discharge. Left ventricle
		<i>per minute</i>	<i>cm.²</i>	<i>cm.²</i>	<i>cm.²</i>	<i>cc.</i>		
VII	Control	84	345	286	59	13.5	6.8	+54
	Insufficiency		359	280	79	21.4	14.7	
	Control after		337	284	53	11.6	5.8	
VIII	Control		350	279	71	18.2	9.1	+39
	Insufficiency		365	279	86	23.8	14.7	
	Control after		348	276	72	18.3	9.1	
486	IIa Normal	120	262	212	50	10.6	5.3	+16
	b Insufficiency immediately		258	207	51	10.9	5.6	
	c Insufficiency later		260	207	53	11.6	6.3	
487	IIa Normal	90	288	249	39	7.3	3.6	+29
	b Insufficiency	89	302	258	44	8.8	5.1	

Furthermore, an undoubted demonstration of increase in diastolic size need not necessarily be interpreted as evidence in favor of regurgitation *during each successive diastole*. For example, it can adequately be accounted for by assuming regurgitation *during a single diastole only*, provided the systolic discharge remains the same.

The results of 14 controlled and carefully evaluated experiments have shown that the systolic discharge of the left ventricle beating at a constant rate may be from 16 to 58 per cent or an average of 36.4 per cent greater during aortic insufficiency than when the valves close normally. Such values give by inference a fair estimate of the percentile regurgitation during each diastole. Two reservations may be thought of however, viz.:

1. The percentile regurgitation may be somewhat less than these figures indicate, owing to the fact that compensatory mechanisms are set into operation which in themselves increase the systolic discharge of the left ventricle (e.g., the increased diastolic size and initial tension of the left ventricle).

2. The percentile regurgitation may be somewhat greater than the

THE MEASUREMENT OF SYMPATHETIC VASOCONSTRICTOR ACTIVITY IN THE LOWER EXTREMITIES¹

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INTRODUCTION

During the last ten years the medical profession has shown a lively interest in the vascular diseases of the extremities. The attitude has changed from a feeling of hopelessness to a desire for more effective therapeutic measures. It has likewise become apparent that accurate diagnosis of the actual condition present in any individual case is often a matter of the greatest difficulty. The work of Allen and Brown (1) has materially helped in untangling the confusion in this group of conditions.

At the present time the majority of our tests indicate only the adequacy or failing of the circulation. The color of the extremity at the horizontal, in dependency, at elevation almost to the vertical, and after exercise is very important. The time of color return after pressure has blanched an area indicates the efficacy of capillary circulation. The blushing test following removal of a tourniquet often tells fairly accurately the level below which the limb is in danger. The oscilometer may show that pulsation is still present though it can no longer be palpated. The salt solution intradermic wheals help us to judge the degree of circulatory impairment. The measurement of surface temperatures by thermocouple readings, and the rapidity of return of such temperatures, after constricting the circulation, give data of value. The only method so far available to estimate the relative amount of vasoconstriction is the intravenous typhoid vaccine reaction

¹ Presented in abstract form at the meeting of the American Society for Clinical Investigation, May 5, 1930.

introduced by Brown (2). This procedure gives a general reaction of considerable severity. If the surface temperatures of the extremities are recorded by thermocouple readings during this time an indication of the amount of vasospasm is evident. If the disease is purely organic obstruction practically no change takes place in this vasomotor index.

It has long been known that there is a vasodilatation under general anesthesia. The loss of heat from this source makes it necessary to keep the patient well covered throughout an operation. This is a general response of the peripheral vascular network. It occurred to us that there might be a vasomotor response following spinal anesthesia. It was relatively easy to test the cutaneous temperatures of the feet of patients under this form of anesthesia.

METHODS AND RESULTS

The first tests were made on a group of patients with normal vascular systems as far as could be determined by the usual clinical examinations.² These patients suffered from such conditions as gallstones, ventral hernias, bilateral inguinal hernias, and chronic appendicitis. They were all in good condition and the operation was in every case an operation of election. Spinal anesthesia was chosen usually for technical advantages. The vasomotor response of these people who were in good general physical condition with normal vessels was almost uniform. Surface temperatures were recorded at identical points on the soles and dorsal surfaces of the feet and on plantar surfaces of the great toes of both sides. The feet were exposed for twenty minutes and the skin temperatures allowed to adjust themselves, several readings being taken to establish a base line. The room temperature was kept as constant as possible at about 23°C., and care was taken to exclude air currents. A constant temperature room was not available. Readings were then made as soon as possible after spinal anesthesia had been induced and at frequent intervals thereafter. The table was

² The surface temperatures were determined by the thermocouple method. An improved apparatus for such measurements was devised and has been briefly described in the *Journal of the American Medical Association*. All of the later determinations were made with this instrument, the *Dermatherm*.

tilted from five to ten degrees within a few minutes after the lumbar puncture.

There is a fluctuation in the surface temperatures of normal feet, at times the readings on the same foot varying as much as 2°C . There is rarely more than one degree in difference between the two sides. The sole regularly has a higher surface temperature than the under surface of the great toe, the former being anywhere from 3° to 8°C . higher than the latter. The average readings for twenty-two cases before opera-

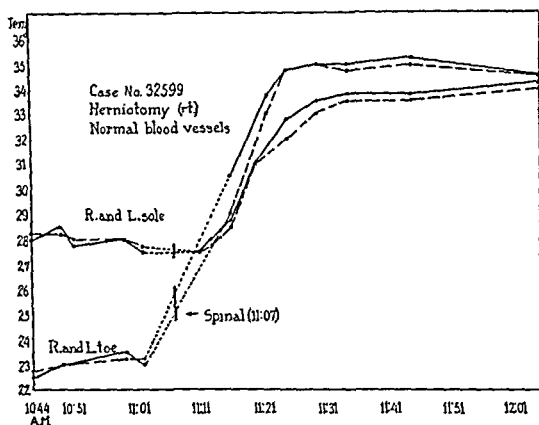


FIG. 1. THE VASOMOTOR RESPONSE OF NORMAL VESSELS FOLLOWING SPINAL ANESTHESIA

tion were $24.7 \pm 3^{\circ}\text{C}$. for the toes and $30.25 \pm 3^{\circ}\text{C}$. for the soles. The height of the peak which the surface temperature reached after spinal anesthesia was almost the same for both soles and toes, $34.2 \pm 2^{\circ}\text{C}$. and $34.5 \pm 1.5^{\circ}\text{C}$. respectively in twenty-two observations. There is thus a much larger proportionate rise for the surface temperature of the toes than for that of the soles after spinal anesthesia. The reading is so constant that it undoubtedly represents the maximum vasodilatation response for the normal vessels of the lower extremities (fig. 1). Thirty-three degrees centigrade is its lower limit in the toe (named by us "normal vasodilatation level"). For purposes of estimating the

element of organic obstruction in a vascular disease the maximum surface temperature reached after sympathetic paralysis is subtracted from this figure and the difference is the "obstruction index."

The chief exceptions so far noted to the above normal reactions are in patients with fever; or in those with advanced carcinoma. Patients with fever have the surface temperatures of the feet already at the maximum vasodilatation level. Patients with advanced carcinoma strangely enough also show high surface temperature readings for the extremities. In three such patients on whom palliative operations

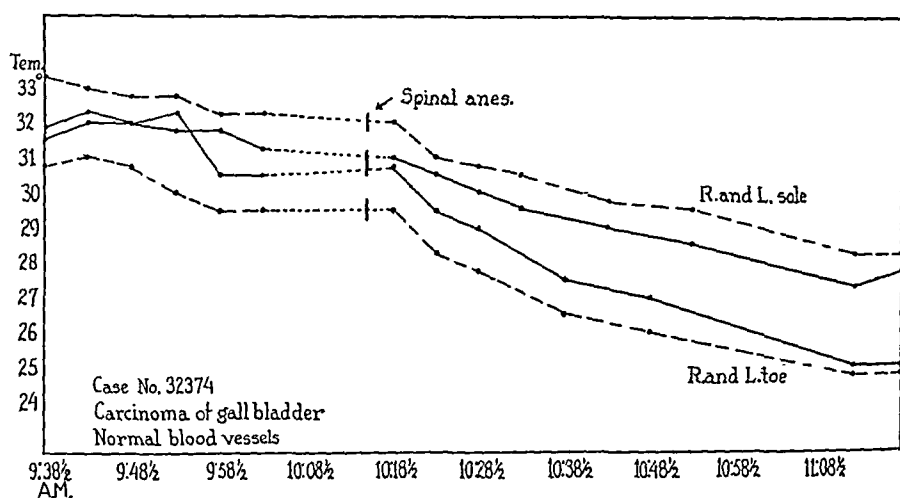


FIG. 2. AN EXCEPTION TO THE RULE FOR NORMAL VESSELS IS SEEN IN ADVANCED MALIGNANCY

were performed under spinal anesthesia, the surface readings before anesthesia registered as an average at 32.5°C. Following spinal anesthesia there was a steady drop of surface readings in two cases so that the feet were from two to five degrees C. colder after operation than before (fig. 2). In the third case there was a faint rise above the preoperative level but it was only transient. The temperatures remained approximately the same after the spinal anesthesia in this case.

We have also had an opportunity to test the response to spinal anesthesia in certain types of vascular disease of the extremities. Tests have been made on cases of endarteritis obliterans with and without vasospasm, on thromboangiitis obliterans with vasospasm, on

arteriosclerotic diseased vessels; but not as yet on true Raynaud's disease. The tests have been made on patients with these conditions both before and after lumbar ganglionectomy, and in some instances where only a unilateral operation had been performed. Cases typical for each group studied will be cited below to illustrate the usefulness of the method.

In a typical case of endarteritis obliterans where pulsation could not be made out below the left femoral artery, but was present though diminished in the vessels of the right foot, the surface temperatures of

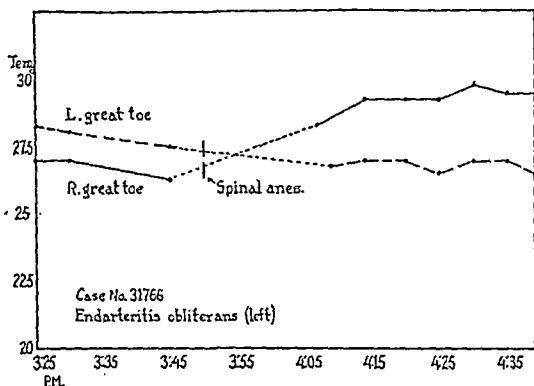


FIG. 3. Diseased vessels vary in their vasomotor reaction depending on the condition. The left toe was the cause of complaint in this patient but even the right side shows only a moderate reaction. Pulsation in vessels of foot—absent on left, feeble on right.

both feet and toes were approximately equal. Following spinal anesthesia there was a drop in cutaneous temperature on the left where the vessels were diseased, and a rise on the right side where the vessels gave evidence of less involvement. It was apparent that vasospasm was not an important factor in this case and that operation on the sympathetic nerves would not be successful. The lesion was probably an obliterative endarteritis on a syphilitic background (fig. 3).

A similar case of endarteritis of syphilitic nature showed popliteal

pulsations on both sides and pulsations in the arteries of the left foot, but no pulsation could be made out in either the dorsalis pedis or posterior tibial artery on the right. The right sole and great toe were colder than the left on thermocouple readings and after spinal anesthesia, although the left foot responded with an abrupt and sustained rise in temperature, the right response was less in degree and slower. The right side reached the normal vasodilatation level but the left side had an "obstruction index" of 2° . This test showed that there

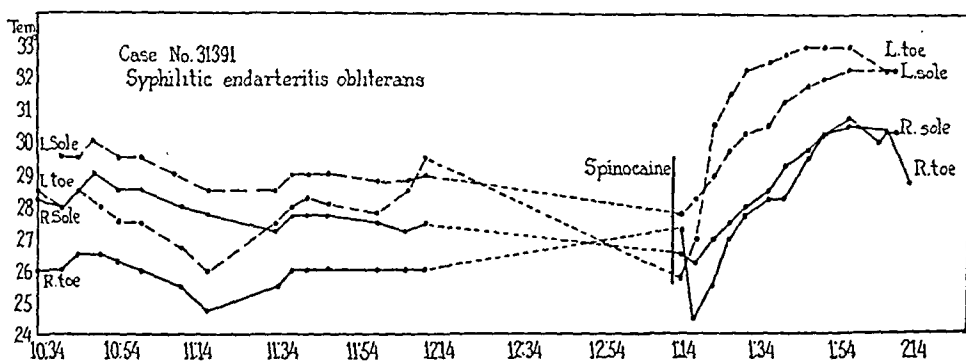


FIG. 4. In this case of endarteritis the right foot caused the trouble. The solid lines are for the right sole and toe respectively and the dotted lines for the left sole and toe. It is apparent that the readings for the right sole and toe are about $2^{\circ}\text{C}.$ colder than the corresponding left readings. After spinal anesthesia the right side shows a fair vasomotor response but not as good as that of the more nearly normal left foot.

was a moderate vasospastic element present together with definite obstruction (fig. 4).

In a diabetic patient with obliteration of the arteries and a previous mid thigh amputation on the left, the question of palliation for impending gangrene of the right foot became urgent. Surface temperature readings indicated that the great toe was warmer than the sole. But after spinal anesthesia the toe showed scarcely any response while the sole gave a decided surface temperature rise. From this study it seemed that the vessels supplying the sole of the foot showed vasoconstriction and reacted to the normal vasodilatation level. The toe, however, showed no vasoconstriction but an "obliteration index" of 2° .

We interpreted this as meaning that the main arterial channels to the toe were completely obstructed. The smaller arteries showed no

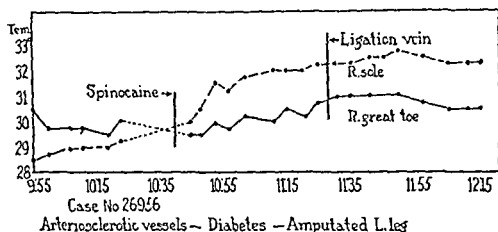


FIG. 5. In this case of arteriosclerosis the surface temperature of the great toe is higher than normal but the response is not marked. The sole shows a much better reaction.

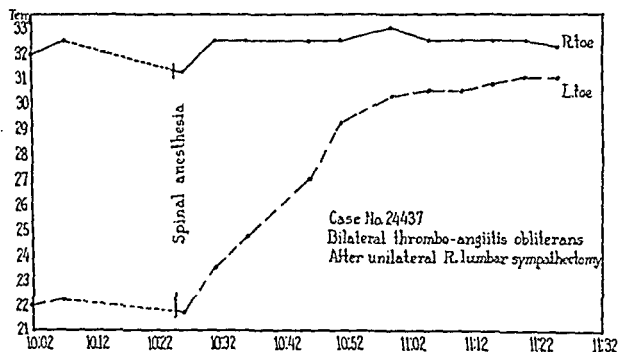


FIG. 6. The contrast between the complete vasodilatation on the right side which has had the lumbar sympathetic ganglia removed and the left unoperated side is striking. The surface temperature response of the unoperated side to spinal anesthesia indicates that there is a large element of vasospasm in the vascular condition on that side.

spasm, possibly due to diminished intravascular pressure. Simple ligation of the popliteal vein was done in the hope of producing better peripheral distribution of the arterial blood (3) (fig. 5).

A patient with characteristic symptoms of thromboangiitis obliterans, more marked on the right, was tested with typhoid vaccine, and gave evidence of vasospasm. Right lumbar ganglionectomy was done with marked lasting relief on the right side. During the year, however, the condition progressed on the left side. This patient presented an ideal case for comparison of the operated and non-operated sides. The surface temperature showed that the sympathectomized limb had high readings on the right sole and great toe as contrasted with those on the left which were 6° to 10°C . colder. When spinal anesthesia was given there was a marked rise in surface temperature on the cold left side while the right side was already within 1° of the normal vasodilatation level and showed no increase. It may be necessary to remove the left lumbar sympathetic chain in order to arrest the progress of the disease on that side (fig. 6). The right side which was much the worse originally is now considerably better than the left. We are studying the surface temperature response to vasodilating drugs in this same patient. There is an excellent opportunity to thus compare the drug action with the known effective paralysis of sympathetic overactivity.

DISCUSSION

Spinal anesthesia acts as an effective temporary chemical block between the spinal cord and the peripheral nerves. It shuts off all motor, sensory and sympathetic impulses and allows the maximum release from their action. Consequently sympathetic inhibition as indicated by peripheral vasospasm is no longer active. This results in a maximum vasodilatation which manifests itself by a uniform rise in surface temperature of the extremities. The surface temperature readings for normal individuals under spinal anesthesia show that there is a close agreement and a narrow range of variation for complete vasodilatation. This furnishes an average surface temperature top-level which may be used as a gauge against the surface temperature readings in patients with abnormal peripheral vessels under similar conditions. By testing various areas of the limb it is possible to judge the degree of occlusion and spasm for the vessels and anastomotic circulation supplying that area. The test is easily carried out in a relatively short period of time which makes it contrast favorably with

Inhalation of nitrous oxide and oxygen combined with surface temperature readings might be of value in estimation of vasospasm in such cases.

This has not been used extensively so far, as we were anxious to use tests of selective and not of general nature.

We hope to further simplify the tests for vasoconstrictor activity by modifying the inhalation method when we have once satisfied ourselves regarding the basic process involved.

Since writing this paper an interesting study by Dr. James C. White (5) has appeared. He also advocates blocking the sympathetic nerves to determine the presence or absence of vasoconstriction.

CONCLUSIONS

The importance of deciding whether a given peripheral vascular disease is due to vasospasm, occlusion of the lumen, or combination of the two is recognized.

Spinal anesthesia is offered as a test which will simplify the differentiation of these elements in the lower extremities.

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SYMPATHETIC INHIBITION OF THE LARGE INTESTINE IN HIRSCHSPRUNG'S DISEASE¹

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Physiologists have known for many years that lumbar sympathetic stimulation caused inhibition of motor activity in the colon together with contraction of the internal sphincter ani (1) (2). No direct application of this knowledge to the treatment of Hirschsprung's disease was however attempted until Royle observed marked improvement in the obstinate constipation of spastic paralytic patients after interrupting the lumbar sympathetic connections (3). Hunter studied the problem in the laboratory (4). Their clinical and experimental experiences that improved motor function of an inert colon followed the removal of the sympathetic influence, furnished the basis for testing the effect of a similar procedure in the treatment of congenital idiopathic dilatation of the colon. This condition had long been suspected of having a neurogenic basis (5) (6). But not until the relief of it achieved by Wade and Royle (7) through lumbar sympathetic ramisection, was there any convincing evidence that the extrinsic nervous mechanism of the bowel was etiologically involved.

Besides this original case, the results of interrupting the sympathetic pathways to the colon have been recorded in six others of Hirschsprung's disease. Wade (8) operated upon four of these patients and Judd and Adson (9) reported two. We are sure that some others have been treated in this way but the results are not available as yet in the literature. In five of the seven cases the results have been eminently successful, while in two they have been unsatisfactory. The failure to obtain uniform results raises the question, in a given case of megacolon what effect will be obtained by operative interruption of its sympathetic innervation. Further information concerning the nature

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and location of the mechanism that seems to be inhibiting normal motor activity in this disease is also desirable. Two typical cases of Hirschsprung's disease recently studied have enabled us to estimate the element of sympathetic overactivity and to trace the site of its origin somewhat further.

CASE REPORTS

Case 1. V. S. (S. M. H. number 23225), a seven year old Italian boy, entered the hospital on account of extreme constipation. He had never had a bowel movement without the aid of enemas and cathartics. On admission his bowels had not moved for two weeks, a period not unusual for him. During prolonged periods of obstipation his abdomen became prominent, and occasionally he had suffered from nausea and foul vomiting. At the age of thirteen months the mother carried out a series of anal sphincter dilatations under her physician's direction without relief.

Examination showed an alert, somewhat emaciated boy, underdeveloped for his age, with a markedly distended abdomen. Both the sigmoid flexure and the cecum were palpable as non-tender masses. On rectal examination the internal sphincter was spastic. The urine was negative; the hemoglobin was 77 per cent; white blood cells 6,450; red blood cells 4,800,000; polymorphonuclears 75 per cent. The Wassermann reaction was negative. The stool was negative. A barium enema on admission (fig. 1) showed an enormous dilation of the rectosigmoid. There was no obstruction.

The clinical diagnosis was a typical example of idiopathic dilatation of the colon (Hirschsprung's disease).

After medical treatment for a month the colon was fairly clean. The significance of the extrinsic nervous mechanism in the clinical picture was then tested in the following manner. A barium enema was given. Four quarts of the solution were injected without causing any discomfort (fig. 2). The patient was then asked to expel as much of the solution as he could, being allowed to sit up for this purpose. By great effort he expelled not over a quart. The width of the colon and the distribution of the barium in it were not materially altered (fig. 3). He was then given spinal anesthesia (1 cc. of spinocaine used). About five minutes after the introduction of procaine, he began complaining of intense paroxysms of itching in the abdomen. These were soon associated with vigorous peristaltic rushes resulting in involuntary expulsion of the barium. In the next fifteen minutes he was able to expel much of the barium solution, almost completely emptying the right and transverse colons and greatly diminishing the dilatation of the sigmoid flexure (fig. 4). He continued to pass more of the barium voluntarily during the next two hours. Another x-ray taken 3½ hours after the beginning of spinal anesthesia (fig. 5) showed how much he had been able to expel. This great augmentation in the motor efficiency of the bowel, temporarily achieved by paralyzing its extrinsic

nervous mechanism, seemed to indicate that permanent improvement would follow the removal of the sympathetic impulses to the large bowel. As both the right and left segments of the colon were involved, bilateral lumbar sympathetic ganglionectomy was performed on October 8, 1929. On October 19, he had the first spontaneous bowel movement in his life and by November 1 was having two to four normal soft formed stools per day. On November 4, another barium enema was



FIG. 1. CASE 1. MEGALOCOLON ON ADMISSION

given to record the motor power of the colon. In the first place about two and a half quarts now produced a sensation of fullness so that he had some difficulty in retaining the enema (fig. 6). When allowed to expel it he evacuated about half of the mixture, almost completely emptying the right colon (fig. 7). Three hours later the striking haustration of the bowel was in evidence (fig. 8). He was discharged from the hospital on November 9, 1929.

January 10, 1930, another barium enema was given which he now had even greater difficulty in retaining. When two quarts had been injected he had a feel-

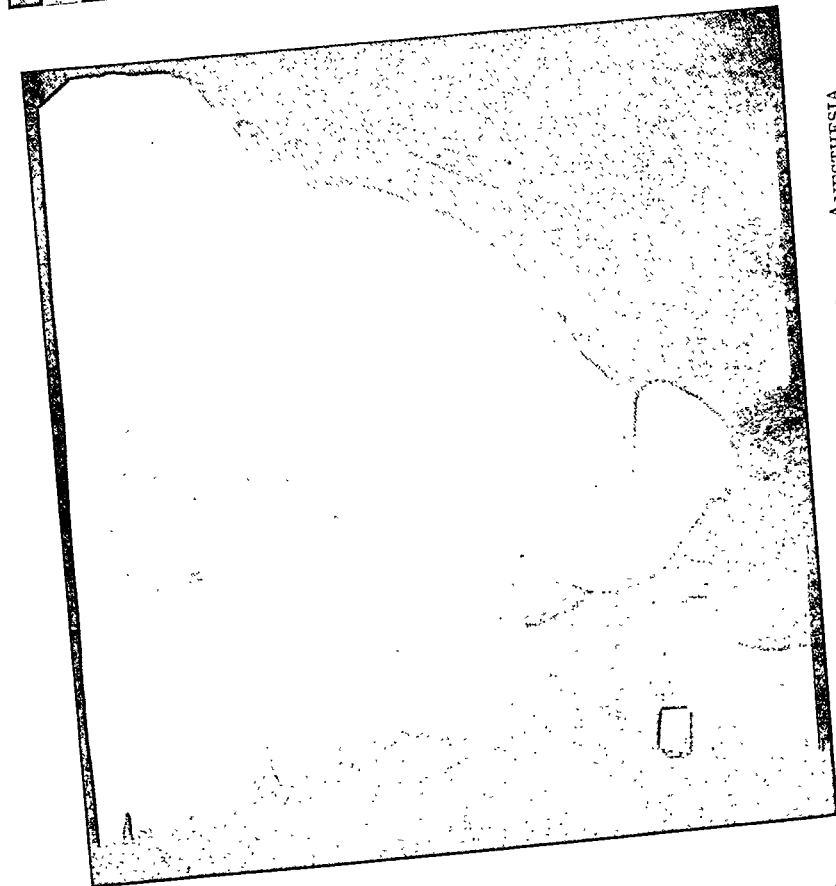


FIG. 2. CASE 1. MOTOR TEST WITH SPINAL ANESTHESIA
Barium enema. Four quarts given without any feeling of fullness.



FIG. 3. CASE 1. MOTOR TEST WITH SPINAL ANESTHESIA
After maximum spontaneous expulsion (about one quarter). No change in distribution, or in diameter of colon.



FIG. 6. CASE 1. FOUR WEEKS AFTER BILATERAL LUMBAR SYMPATHECTOMY
Two and one-half quarts cause a feeling of fullness.



FIG. 7. CASE 1. FOUR WEEKS AFTER BILATERAL LUMBAR SYMPATHECTOMY
After spontaneous expulsion—ascending and transverse colons nearly emptied.

ing of great fullness (fig. 9). When allowed to evacuate the enema he expelled every trace of barium (fig. 10).

Since November 1, 1929 (three weeks after operation) to the present (six months) he has been having regular daily spontaneous bowel movements usually twice a day, and has gained five pounds in weight since his operation.



FIG. 8. CASE 1. FOUR WEEKS AFTER BILATERAL LUMBAR SYMPATHECTOMY

Four hours later. Spontaneous evacuation has expelled most of the enema. Note the excellent haustration in transverse colon.

Case 2. R. L. (S. M. H. number 32554), a five year old boy, had been constipated since birth. In the first two weeks of his life only one bowel movement was obtained. His local physician, thinking that the sphincter might be tight, instructed the mother to dilate it every day. This maneuver did not, however, result in better evacuations. He has since then only twice had a desire to defecate and has not had a bowel movement without the aid of cathartics and enemas. His mother by constant attention, by giving large doses of cathartics and frequent



FIG. 9. CASE 1. THREE MONTHS AFTER BILATERAL LUMBAR SYMPLECTECTOMY

Two quarts now cause discomfort and urgency

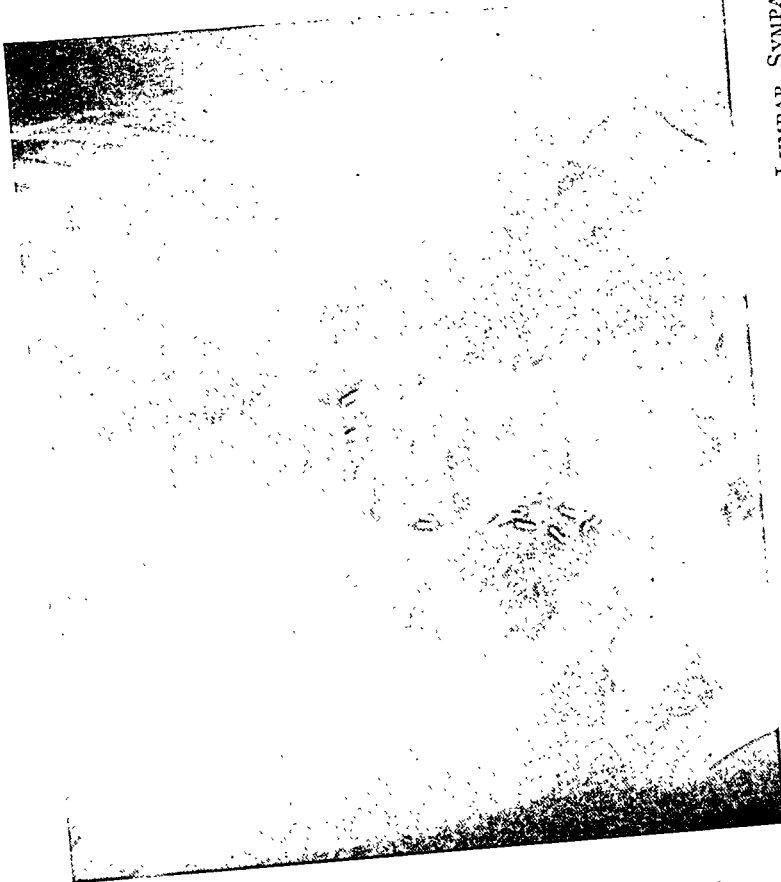


FIG. 10. CASE 1. THREE MONTHS AFTER BILATERAL LUMBAR SYMPLECTECTOMY

Complete spontaneous expulsion of the barium. Silver clips indicate extent of lumbar sympathetic chains resected.

enemas has succeeded in preventing obstructive vomiting. Nine days frequently passed nevertheless without a bowel movement. His appetite would become poor, his abdomen greatly distended. When his bowels moved he passed enormous amounts of material.

Examination showed an alert, well-developed youngster. His mucous membranes appeared to be slightly pale. When he stood erect his abdomen was promi-

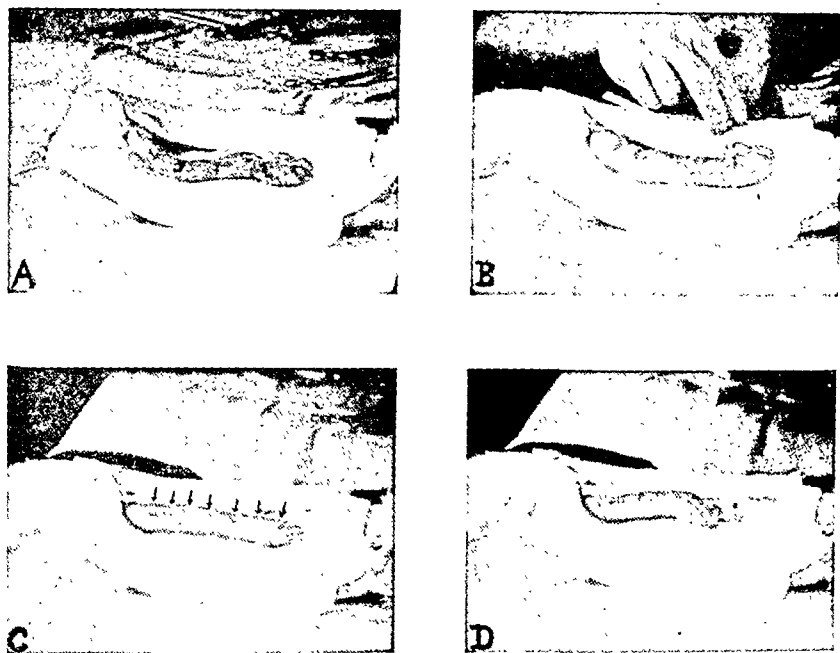


FIG. 13. THE EFFECT OF SPINAL ANESTHESIA ON THE MOTOR ACTIVITY IN THE LARGE INTESTINE OF THE CAT

- A. After exposing and walling off the colon it is flabby and inert.
- B. Even distending it with saline causes no peristalsis and no emptying.
- C. Coördinated peristalsis begins almost immediately upon the induction of spinal anesthesia. Arrows indicate peristaltic waves seen in the moving film.
- D. Contractions of both longitudinal and circular fibers cause shortening and emptying of the distended colon after spinal anesthesia.

nent. In the recumbent position after a bowel movement, when his abdomen was relaxed, diastasis of the rectus muscles became evident. The abdominal wall was thin and flabby, due apparently to periodic distension. The internal sphincter ani was tight and the rectal ampulla large and empty. Examination was painless. The urine was negative. There were 4,540,000 red blood cells; the hemoglobin was 75 per cent; the leucocytes numbered 7,850; the Wassermann reaction was negative.

His markedly dilated colon held three and a half quarts of barium solution (fig. 11). The abdomen became very prominent but he experienced no discomfort. After fifteen minutes of effort he was unable in the horizontal position to expel any. Spinocaine, 0.8 cc. diluted during injection with spinal fluid to 2.2 cc. was injected intraspinaly. In about twelve minutes he became anesthetic to the level of the umbilicus and hypoaesthetic well above the costal margin. He had also lost the ability to move his toes. He complained of itching of the abdomen and scratched vigorously. When the sensory and motor symptoms first began to appear he became nauseated and simultaneously evacuated a large quantity of the barium enema. At the same time he vomited a small amount. After several evacuations of rectal contents his abdomen became relaxed. Fifteen minutes after the beginning of the spinal anesthesia another x-ray was taken which showed that most of the barium enema had been expelled. The ascending and transverse colons were nearly empty. The sigmoid flexure still contained a moderate amount but its diameter was greatly reduced (fig. 12).

The results of this test convinced us that sympathetic overactivity played the predominant rôle in this boy's disease and that interrupting sympathetic impulses would result in permanent improvement. He has not yet been operated upon.

In these two typical cases of Hirschsprung's disease spinal anesthesia temporarily terminated the motor inertia of the large bowel. In order to test the efficiency of this procedure experiments were carried out in animals. The colon was exposed in ten cats by opening the abdomen under light ether anesthesia. The small intestine was packed off with gauze. The surface of the colon was kept moist by a saline spray. Under these conditions the colon was always found to be inert. If its wall was stimulated by pinching, a small local contraction occurred. Coordinated peristalsis of the large intestine was observed in only one of the ten cats. In this animal (a pregnant cat) on first opening the abdomen a few feeble, ineffective peristaltic waves were seen, but they ceased entirely within three minutes. Distending the colon with saline solution through the ileocolonic valve in no case caused any peristalsis or evacuation of its contents. Profound asphyxia was the only state in which motor activity took place when the extrinsic innervation remained intact.

If however, the paths of the sympathetic nerves to the large bowel were interrupted at any point, vigorous motor activity promptly appeared. If both lumbar sympathetic chains or the inferior mesenteric ganglia were removed, then peristalsis and increased muscular tonus was widespread. But, if one or more of the colonic nerves in

the mesentery of the colon were cut distally to the inferior mesenteric ganglion, then motor activity appeared only in the segment supplied by that nerve. Evidently there is constant sympathetic stimulation passing to the colon under the conditions of the experiment (probably a reflex from the peritoneal wound) completely inhibiting motor activity until the sympathetic pathway is interrupted. Spinal anesthesia was followed in every instance by the onset of motor activity fully as marked as followed complete mechanical interruption of the sympathetic fibers to the colon. A small area of the dura was exposed in the lower lumbar region. Through a fine 27 gauge right angled needle as much spinal fluid was aspirated as possible. Ten per cent procaine (usually in the form of spinocaine, Metz) was injected into the subarachnoid space, the foot of the table being elevated five degrees. Vigorous peristalsis and anti-peristalsis began in the colon, usually within five minutes, followed very quickly by shortening and narrowing of the whole large bowel. If the colon had been distended with fluid before the induction of spinal anesthesia, motor activity brought about evacuation of its contents. A motion picture film shows this sequence of events. The four stages of this process are shown in figure 13, taken from this film. If the attempt was made during spinal anesthesia to inject fluid into the colon it could not be distended as it could easily have been before paralyzing the sympathetic innervation. At about the time that motor activity appeared in the colon it was found that the muscular tonus of the lower extremities was lost while that of the upper extremities was unaffected. In other words, procaine had resulted in paralysis of the lumbar motor nerves to striated muscles and of the sympathetic inhibitory nerves to the large intestine. We conclude from these experiments that spinal anesthesia is a satisfactory method of overcoming temporarily sympathetic inhibition of the colon.

DISCUSSION

Spinal anesthesia as a test of sympathetic overactivity. Pioneer efforts have opened up great possibilities in several different fields for the removal of an excessive sympathetic stimulation. The greatest need in this development appears to be accurate criteria to demonstrate whether sympathetic overactivity is present and, if so, what will be

the effect of its removal. For this purpose temporary abolition of sympathetic stimulation seems a priori the most reasonable procedure, and in practice has given us excellent results in the gastro-intestinal and the vascular systems.

In the two clinical cases of Hirschsprung's disease we have demonstrated the immediate augmentation in motor activity of the large bowel upon the induction of spinal anesthesia.² In one of these cases it has been further shown that such temporary improvement in function has been made continuous by the removal of the lumbar sympathetic chains. The animal experiments have shown that spinal anesthesia is entirely effective in overcoming sympathetic inhibition of the colon. We propose the adoption of this procedure to ascertain in any individual case of megalocolon how effective operative interruption of its sympathetic innervation will prove to be.

The nervous mechanism involved. These observations have advanced somewhat farther our knowledge of the mechanism underlying the motor anomaly of Hirschsprung's disease. The previous results of operations on the sympathetic nervous system (Wade and Royle (7), and Judd and Adson (9)) have made it clear that there is probably sympathetic overactivity in this condition. But both types of operation employed by them, ramisection and ganglionectomy, trace the origin of the sympathetic influence with certainty only to the lumbar ganglia and do not establish involvement of the central nervous system, although both reports quoted express the opinion that the latter is implicated. The effectiveness of spinal anesthesia makes clear, however, that motor inactivity in at least one type of Hirschsprung's disease is dependent upon central sympathetic inhibition of the large bowel. Whether sympathetic stimuli to the colon arise from impulses originating in the cord at higher levels or reflexly from sensory stimuli in the splanchnic area cannot be decided upon the basis of our present information. Experimentally in animals both types of sympathetic inhibition of the colon have been demonstrated; from higher centers by Hunter (4), and in the case of the peritoneal cavity by our experiments. The information at our disposal does not even permit the inference that the actual number or strength of sympathetic efferent

² In choosing the dosage in children of various ages we have followed the table worked out by Pitkin (16).

impulses in this disease is greater than normal, since it would be possible to account for the known facts on the basis of peripheral hypersensitivity to normally acting, constant sympathetic stimulation. This explanation seems however less likely. In any event, the effectiveness of spinal anesthesia in permitting motor activity of the bowel affords strong evidence for the belief that the anomaly in our cases of Hirschsprung's disease is not due to permanent injury to or defect in the neuro-muscular mechanism such as degeneration in Auerbach's plexus as described by Cameron (10). It also shows that efferent stimuli initiated in the central nervous system and traveling over the lumbar sympathetic pathways to the large bowel play a leading rôle in the mechanism. Whether the sympathetic influence is chiefly exerted through the inhibition of the muscular coats of the colon or through contraction of the sphincters (internal sphincter ani and O'Beirne's) is another point that we have no way of deciding at present. Sympathetic stimulation brings about both of these results, (Bayliss and Starling, (2) and Learmonth and Markowitz (11)) and probably both of them play a certain part. The peristaltic rushes observed under spinal anesthesia suggest that the activity of the intrinsic musculature was at least as important as the relaxation of sphincters.

Results of sympathectomy. As eminent an authority on the surgery of the autonomic nervous system as Kappis (12) stated in 1929, "Tierny³ made the proposal to divide the rami communicantes of L 1 and L 2 in megacolon and so to decrease the spastic tonus; he will probably not find many imitators." In view of the small number of cases of Hirschsprung's disease so far treated by sympathetic operations it may be well to call attention to the extremely promising results that have been achieved. Our case adds another to this small group. When he came to us, this seven year old boy had never had a spontaneous bowel movement without the aid of strong catharsis and enemas. Since operation (six months) he has continuously had normal daily evacuations without assistance.

The operation of choice at the present time we believe is lumbar sympathetic ganglionectomy as devised by Adson (15). Eventually

³ Tierny (13) reviewed an article by Bartle (14) who in 1926, on the basis of Royle and Hunter's observations, advised sympathetic ramisection for Hirschsprung's disease before any of the results of this procedure had been reported.

it will probably be feasible to interrupt the nervous pathways to the colon in the region of the inferior mesenteric ganglion, without interfering with other pathways in the lumbar sympathetic outflow. At present, assurance of complete interruption of stimuli to the large bowel is more important than preserving the sympathetic innervation of the extremities. If this test is used to select appropriate cases and if an operation certain to permanently interrupt the entire sympathetic innervation of the large bowel is chosen we feel confident that the results of the latter will be most satisfactory.

CONCLUSIONS

1. At present a most important desideratum in surgery of the sympathetic nervous system is the development of accurate criteria for detecting sympathetic overactivity in the various systems affected.
2. The temporary interruption of sympathetic stimuli to the involved area is used to select appropriate cases and to estimate how much effect removal of sympathetic innervation will have.
3. Spinal anesthesia has produced temporarily motor activity of the colon in two cases of Hirschsprung's disease and in experiments in animals.
4. The motor anomaly in at least one type of megalocolon is dependent upon central sympathetic inhibition of the large bowel.
5. We propose the effect of spinal anesthesia upon the motor function of the large bowel as a test of sympathetic overactivity in Hirschsprung's disease.

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THE SERUM CALCIUM IN POLYCYTHEMIA VERA

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In a study of fourteen cases of polycythemia vera, Brown and Roth (1) found the serum calcium to be over 11.0 mgm. per hundred cubic centimeters in every case. The actual range of serum calcium values was 11.1 to 18.1 mgm. per hundred cubic centimeters. The average value for the group was 14.3 mgm. These authors noted a reduction in

TABLE 1
Data on patients

Case number	Sex	Age	Hemoglobin (Sahli)	Red blood cells	White blood cells	Serum calcium	Spleen palpable
			per cent	millions		mgm. per 100 cc.	
1	M.	54	107	7.5	20,300	10.0	+
2	M.	53	132	9.7	13,000	10.7	+
3	F.	63	125	7.0	16,200	7.9	+
4	M.	44	128	6.6	15,200	11.0	0
5	F.	60	119	7.8	14,700	10.8	+
6	M.	47	160	10.3	7,800	12.9	+
7	M.	47	102	6.9	11,600	10.7	+
8	M.	46	125	9.0	9,500	9.9	+
9	M.	57	128	8.9	15,200	11.3	+

serum calcium accompanying a fall in red blood cell count following treatment with phenylhydrazine.

We were stimulated by this report to make some additional studies of the calcium metabolism in polycythemia, but the work was halted in its incipency by our inability to confirm the findings of Brown and Roth.

Serum calcium determinations were made in nine cases of typical polycythemia vera using Clark and Collip's modification of the method of Kramer and Tisdall (2). The patients received no special diet, and

in only one (case 6) was phenylhydrazine administered before the first calcium determination was made. The results are shown in table 1.

From this tabulation it can be seen that in only two of the nine cases was the serum calcium in excess of 11.0 mgm. (12.9 and 11.3 mgm.) while in one case it was considerably less than 9.0 mgm. (7.9 mgm.). The remaining six cases all showed perfectly normal serum calcium values. The average for the group was 10.6 mgm.

From these findings we conclude that there is no significant variation in the serum calcium in polycythemia vera.

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SERUM ELECTROLYTE STUDIES IN NORMAL AND PATHOLOGICAL CONDITIONS: PNEUMONIA, RENAL EDEMA, CARDIAC EDEMA, UREMIC AND DIABETIC ACIDOSIS

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In the past ten years there has been an increasing interest in the electrolyte structure of the blood. This interest grew from a realization of the impossibility of interpreting isolated changes in single constituents. Methods for partitioning the anions developed with much greater rapidity than did a similar approach to the cations. In 1923 the authors published with Loeb and Palmer a series of studies on the serum acids including a few base partitions. Although the acid determinations were quite consistent, base results were unsatisfactory due to the unreliability of the sodium and potassium methods. With the development of an accurate method for estimating total cations, a more satisfactory understanding of the total electrolyte structure is possible. This method has evolved slowly and in our hands has not been considered reliable prior to the past three years, when our data became sufficiently consistent to report. We have discarded most of our work before that time.

Even before an acceptable method for total base appeared, Van Slyke and co-workers filled the only essential remaining gap in anion analysis by determining a formula for base bound to protein. It is now possible, therefore, to arrive at a fairly accurate picture of the total electrolyte distribution in serum. It is proposed in this paper to discuss the results obtained by studies carried out on normals and patients with pneumonia, nephritic acidosis, nephritic edema, and diabetic acidosis. A few observations during diuresis are included.

METHODS

The methods employed are those described in a previous paper (1). We are using the formula for base bound to protein published by Van Slyke and associates in 1928 (2). There remain, however, two points to be discussed; first, the technique of blood removal, and, second, the limit of error in the methods.

A tourniquet was used in most instances. The duration of stasis, however, was so short that the order of magnitude of the changes caused by it does not approach that of the alterations caused by disease. Moreover, 60 cc. of blood were removed rapidly through a large needle decreasing thereby still further the possibility of changes due to stasis. Table 1 shows, in normals, the negligible effect of a tourniquet properly used. Since the conditions in which we were most interested are often associated with an extremely low blood pressure, it is practically impossible to draw blood without increasing venous pressure. Also, those particular conditions are characterized by a concentrated blood, requiring more blood to provide the usual amount of serum. Unless the supply of material for analysis is adequate for duplicates and provides a slight surplus for accidents, the whole study may be wasted; for it must be emphasized that our particular interest is in the balance of acid and base as well as in individual ionic variations. Each experiment, therefore, should include a measurement of every component.

A critical examination of the various methods employed brings greater satisfaction with the bicarbonate analysis than with any other method. This is accurate and reliable to a high degree, both in the mechanical procedures and the calculation of resulting milliequivalents. Chloride also causes few misgivings. Phosphate analysis is relatively easy and whatever uncertainty there may be regarding calculations is minimized by the small total phosphate content of serum. The high percentage of error in sulphate analysis on normal blood becomes insignificant when it is recalled that total sulphate is usually less than 1.0 m.Eq.; as sulphate increases, accuracy improves so that high figures can be accepted to within 1.0 m.Eq. We find the sulphates important only in renal insufficiency (where they are elevated). There are two possible defects in dealing with base bound to protein. The formulae are based on data so difficult to obtain that they undoubtedly will be altered by further study, and the albumin-globulin ratios are, in our hands, unreliable determinations. We have assumed Van Slyke's "normal" ratio $\left(\frac{1.6}{1}\right)$ for all bloods. A maximal and unusual error, such as would occur in a case of renal edema with a very abnormal ratio, is 1.0 m.Eq. The influence of clinical changes in hydrogen ion concentration is too slight to be significant. A constant pH of 7.35 is, therefore, assumed. At this reaction the simplified formula is protein m.Eq. = $P \times 2.36$ (P = protein per cent of serum). It is safe to say that base bound to protein is at the present time the most unreliable estimation on the acid side. Base bound to ketones leaves much to be desired in the translation of grams per liter to milliequivalents and we offer our figures as approximations only.

Total base is a far more important determination than any single acid, because as the term implies, it, alone, gives an idea of the amount of base present in the serum, and serves the further important function of delimiting the level of total electrolytes. It is a treacherous method, requiring constant use of the reagents with frequent blanks to prevent occasional errors. No method in clinical chemistry requires more vigilance to make it acceptable. It is still the weak link in any such study as the present one; a fact much under emphasized in the literature of this field. The limit of error of the total base method under the best conditions is ± 2.0 m.Eq., whereas the total error in the various anions is approximately ± 1.7 m.Eq.

NORMALS

Table 1 requires no explanation. Attention is called to the relatively insignificant effect of the tourniquet and to the unimportance of brief exercise. Duplicate observations on single individuals within a few hours agree in all details. A much larger group would be required before one could give reliable averages. The anions need no comment. Total base, however, is surprising in its narrow range between maximum and minimum (148.4 to 155.1 m.Eq.) and its low mean 151.9 m.Eq. For example, Peters, 1926, (3), finds an average of 155.7 m.Eq. and in 1929, (4), 153.8, and Sunderman, Austin and Camac, (1926), (5), give 154.7 m.Eq. Darrow and Hartmann, 1929, (6), however, on children and adults find 150.7 m.Eq. The more recent results reveal lower rather than higher concentrations.

Undetermined acid or total base minus total acid ($B-A$) is low. We do not find large quantities of acid that must be called "organic acid." Our usual figures would be covered by the amounts of lactate and sulphate normally present in serum (1 to 2 m. Eq.). This differs strikingly from the earlier results reported by Peters. Recently he gives values for "organic acid" which more nearly resemble ours. This difference would seem to be due largely to lower total base concentrations.

PNEUMONIA

The literature on chemical studies in pneumonia, prior to the past few years, was well covered by Sunderman, Austin and Camac in 1926 (5). The present authors with Loeb and Palmer in 1923 (7) found during this disease: (1) decreased chloride; (2) normal bicarbonate; (3) low serum protein (by Kjeldahl); (4) decreased freezing point

TABLE 1
Normals

Normals

Date	Initials and sex	Serum										Remarks
		Non-protein nitrogen	Serum protein	Total base	Total acid	B - A	Chloride	Carbonate	Phosphate	Protein		
		mgm. per 100 cc.	per cent	m. Eq. per liter	m. Eq. per liter	m. Eq. per liter	m. Eq. per liter	m. Eq. per liter	m. Eq. per liter	m. Eq. per liter		
May 14, 1926	R. F. L. Male	24	7.4	149.0	148.8	0.2	100.8	29.1	1.4	17.5	Tourniquet	
February 15, 1927		30	7.9	151.9	149.4	2.5	102.6	26.6	1.7	18.6	Without tourniquet, subject fasting	
March 1, 1929	D. W. A. Male	34	7.6	155.2	155.1	0.1	102.6	32.6	1.5	18.4	Without tourniquet	
January 25, 1927		27	7.2	152.3	154.4	-2.1	104.4	30.7	2.3	17.0	After exercise—without tourniquet	
January 25, 1927			7.4	152.9	153.0	-0.1	104.4	28.8	2.3	17.5	Without tourniquet	
March 4, 1927		24	7.5	151.2	152.5	-1.3	103.0	30.0	1.8	17.7	Without tourniquet	
March 4, 1927			7.5	151.4	151.6	-0.2	103.0	29.1	1.8	17.7	Without tourniquet—usual minimum time	
February 14, 1929	R. H. Male	24	7.0	153.2	150.9	2.3	104.2	28.4	1.8	16.5	Without tourniquet	
February 25, 1927		17	7.6	149.8	152.6	-2.8	105.6	26.8	2.1	17.9	Without tourniquet	
January 27, 1927	W. W. P. Male	42	7.5	151.8	151.8	0.0	104.2	28.6	1.3	17.7	Without tourniquet	
January 27, 1927	W S. L.	28	7.3	149.5	152.5	-3.0	105.6	28.2	1.5	17.2	Without tourniquet	

February 15, 1929	G. G. Female	25	7.1	152.3	152.9	-0.6	106.6	27.7	1.9	16.7	
January 21, 1929	Y. K. Male	(30)	7.5	156.7	152.9	3.8	105.0	28.6	1.6	17.7	
March 1, 1929	K. T. Male	30	7.5	152.0	150.8	1.2	100.8	30.8	1.3	17.9	
March 9, 1929	J. M. Male	26	7.2	153.0	152.1	0.9	104.0	29.3	2.0	16.8	
March 10, 1929		26	7.2	152.5	152.9	-0.4	104.0	29.8	2.0	17.1	
Mean.....				151.9	151.8	+0.1	103.5	29.0	1.8	17.6	

tioned above) to account for the striking changes in the blood serum occurring in this disease.

In summary, electrolyte partitions in pneumonia and during convalescence show:

(1) Decrease in total base, protein, chloride and phosphate with slow and roughly parallel return to normal following the crisis.

(2) No increase in undetermined acid ($B - A$) at any stage of the disease.

RENAL EDEMA

Classification of these cases is difficult, but 70979, 81971, 236030 and 84788 are fairly typical uncomplicated cases of renal edema or nephrosis; in other words, simple edema without real hypertension, vascular changes or decreased general renal function. Case 61957 probably should not be included because her previous history of acute nephritis and death three years later in uremia place her in the group currently designated as chronic glomerular nephritis. At the time of observation her clinical picture was so similar to nephrosis that we included her data in the group.

The low percentage of protein in renal edema has been recognized for years and Epstein's original conception of its causal relationship is more and more widely accepted as new data appear. The low protein concentrations to be found in table 3 are consistent with previous work.

Two frequent but not constant alterations in the electrolyte structure appear: first, a decrease in the total base, and second, an elevation of chloride. Only one base in ten was above our normal average and there were no very high concentrations, such as observed by Peters, et al (10). As their high total base values all occurred before 1927 it is possible that the difficulties in the method before that time may account for the discrepancy. The concentrations of phosphate in our cases were within normal limits. This is the only disease in which such high chloride concentrations have been observed either individually or in average (105.6 m.Eq.). However, some cases have values within normal limits without differing clinically from the individuals with high chlorides, nor can one correlate the ordinary course of the disease with the electrolyte levels.

The amount of undetermined acid ($B - A$) was not abnormal; the

very high negative results (i.e., excess of acid over base) found by Peters did not occur in our briefer series. Our cases were in the stage of relatively normal general renal function, so that retention of phosphates and sulphates did not appear. The changes, then, that occur with simple renal edema are: (1) decreased serum protein, an essential and consistent alteration; (2) decreased total base which is usually present; and (3) fairly frequent great increases in chlorides, in most instances with relative decrease in bicarbonate. The importance of salt (NaCl) restriction as a causative factor in decreasing total base in these conditions was not studied. The alterations enumerated above occur without any abnormalities in the acid base balance of the serum and without the slightest relationship to the amount of edema (excepting, of course, the constant parallelism between edema and serum protein content).

An attempt to interpret from available serum analyses the nonprotein factors influencing renal edema, leads at once to the opinion that chloride retention is the most striking and important variation. Not only is the chloride ion increased, but the response to introduction of chloride in any form is a striking elevation of the chloride concentration of the serum (except when vigorous diuresis occurs). The authors with Palmer and Loeb (11) in 1923, administered NaCl to a patient suffering from renal edema; Linder, in 1926, (12) gave HCl; and Peters and co-workers, in 1929 (*loc. cit.*), gave NH_4Cl , all results agreeing in that extraordinary increases of serum chloride were observed. Albright and Bauer (13) have recently published a careful study of the effect of NaCl, NH_4Cl , and NaHCO_3 ingestion in a case of nephrosis. NaCl dosage increased both serum base and chloride, whereas NH_4Cl , causing a diuresis, decreased them. On the other hand, urine studies and clinical observation point to base difficulties. Linder, for example, made the significant observation (in one case) that, whereas normal individuals respond to administration of HCl by increased excretion of BCl and NH_4Cl , the hydremic nephritic responds by increased elimination of NH_4Cl , holding back base while excreting chloride. This result, together with the well-known clinical difference between the effects of NaCl and NH_4Cl administration, indicates that it is impossible from serum studies alone, no matter how complete they may be, to affirm or deny the truth of the current hypothesis

TABLE 3
Renal edema (nephrosis)

Renal edema (nephrosis)

Date	Initials, hospital number and sex	Case number	Serum										Edema	Remarks
			Nonprotein nitrogen	Serum protein	Total base	Total acid	B - A	Chloride	Carbonate	Phosphate	Protein			
February 25, 1926	J. L. 61957 Female	1	33	4.5	153.0	149.9	3.1	112.0	24.8	(2.5)	10.6	+		A girl of 29; acute nephritis following scarlet fever as a child with steadily progressive chronic nephritis, dying in uremia in 1929. In edematous stage when blood was taken
November 22, 1927	70979 Male	2	58	3.9	144.6	144.3	0.3	101.4	31.8	1.9	9.2	++		Steadily progressive renal edema in man of 61. No general impairment of renal function. Typical nephrosis
December 6, 1927			40	3.9	144.9	144.6	0.3	103.0	30.1	2.3	9.2	++		
January 21, 1929			33	3.2	146.3	144.4	1.9	103.2	31.8	1.8	7.6	+++		
May 9, 1929	81971 Female	3	41	3.6	149.1	142.8	6.3	103.8	26.1	2.8	8.6	++		A typical nephrosis of 35 with good general renal function. Spontaneous diuresis occurred between samples
June 28, 1929			22	4.5	145.8	143.2	2.6	106.0	24.6	2.0	10.6	0		
December 19, 1929	84878 Female	4	76	4.5	150.8	148.8	2.0	112.0	24.1	2.1	10.6	+		A woman of 34 with 1 year's history of edema, good general renal function. Blood Pressure 135/80, Heavy albuminuria. She had two transfusions between second and third samples. Her serum protein in July 1929 was 3.9 per cent and she had general edema; in October it was 3.7 per cent with even more edema
January 16, 1930			25	4.3	147.7	146.8	0.9	111.4	23.4	1.9	10.1	+		
February 3, 1930			40	4.9	151.3	148.6	2.7	107.4	27.1	2.5	11.6	0		

December 20, 1929	236030	5	33	3.6	147.3	143.5	3.8	102.0	30.9	2.0	8.6	++	A girl of 23 with 7 weeks' history of edema; without hypertension; excessive albuminuria; good general renal function. She had 20 grams urea daily from January 6 to February 4 with no effect on weight; she had NH_4Cl from March 3 to 12 (12 grams a day) without diuresis; transfusion 600 cc. on February 13
January 6, 1930	Female		35	4.2	147.4	141.9	5.5	100.8	28.5	2.8	9.8	++	
February 7, 1930			46	4.2	139.9	138.8	1.1	96.6	29.8	2.4	10.0	++	
March 12, 1930			40	3.8	145.5	145.0	0.5	110.4	22.5	3.1	9.0	++	



FIG. 3.—Case 236030

attributing importance to sodium in the production of edema (Blum). Base and acid balance studies are very difficult in any patient, but particularly so with the albuminous urine of nephrosis; such an approach, however, seems of prime importance.

The effect of a low value for serum protein on the distribution of chlorides between blood stream and tissue spaces according to the Donnan equilibrium, is not negligible and would tend to increase serum chlorides; it would also cause a greater elevation of serum chloride if chloride intake were increased. This, of course, is dependent on the fact that the greater the difference between serum protein and tissue fluid protein, the greater is the excess of chloride in the latter fluid over that in the corresponding serum. Therefore, the closer the two protein concentrations approach each other (i.e., as serum protein decreases or tissue fluid protein increases) the higher will be the serum chloride level without any increase in total body chloride. Moreover, the addition of a definite amount of BCl to the body in such cases would produce, *ceteris paribus*, relatively higher increases in serum figures than would similar amounts in normals. This factor may explain in part the unusual serum chloride results in nephrosis where the concentration of serum protein is consistently low.

Blood volume studies in renal edema would supply a needed factor for proper understanding of the fluid and electrolyte changes. Bock, in 1921 (14) followed blood volume during diuresis in cardiac and renal edema, and discovered that the nephritic patient lost plasma volume and body weight proportionately so that there was no change in the ratio $\frac{\text{plasma volume}}{\text{body weight}}$. The cardiac case, however, maintained his plasma volume practically constant with a striking increase in plasma volume per kilo. This may indicate that all body fluids are equally involved in the disturbance of water balance in the true edematous nephritic, whereas the edema of vascular stasis involves mainly the tissues.

DIURESIS

In the papers with Loeb and Palmer, cited above, we called attention to a change in the relationship between the chloride and bicar-

TABLE 4
Diuresis

Date	Hospital number and sex	Case number	Serum								Remarks	
			Nonprotein Nitrogen	Serum protein	Total base	Total acid	B - A	Chloride	Carbonate	Phosphate		Protein
			mgm. per 100 cc.	per cent	m. Eq. per liter	m. Eq. per liter	m. Eq. per liter	m. Eq. per liter	m. Eq. per liter	m. Eq. per liter	m. Eq. per liter	
April 17, 1926 April 20, 1926	60406 Male	1	41	5.4	144.3	144.3	0	100.0	30.3	1.7	12.3	A cardiac of 53 due to chronic vascular changes in myocardium. Before diuresis and during diuresis with diuretin
			26	6.0	146.0	146.4	-0.4	99.2	31.8	1.6	13.8	
April 27, 1926 May 5, 1926	65651 Female	2	48	6.8	148.8	147.1	1.7	102.8	26.4	2.0	15.9	A cardiac edema of 34 due to mitral disease. Blood taken before diuresis, and after diuresis. Lost 13 kgm. of weight
			25	7.2	148.3	141.8	3.5	92.2	33.9	1.7	17.0	
January 10, 1927 10:45 a.m. 3:45 p.m.	41962 Female	3	21	6.6	147.0	143.7	3.3	94.4	32.5	1.8	15.0	2 mgm. novasurol at 10:50 a.m. with marked diuresis. A cardiac of 69 with mitral and aortic disease
			(21)	6.0	147.0	141.1	5.9	91.8	33.8	1.8	13.7	



FIG. 4—Case 65651

bonate concentrations following cardiac diuresis. This consisted of a striking decrease in the former with a coincident increase in bicarbonate. Because of the constancy of conductivity it was assumed that no appreciable change in total electrolytes took place, and the lack of variation in the serum protein made great changes in dilution unlikely. We have repeated this observation with a complete electrolyte partition in case 65651 (table 4). A drop of 10.6 m.Eq. in chloride was accompanied by an increase of 7.5 m.Eq. in bicarbonate and 1.1 m.Eq. in protein, but base remained constant, i.e., total electrolytes did not change. The decrease in chlorides paralleled loss of edema more obviously than did fixed base. In the previous section it was seen that chloride was often elevated in renal edema; here, as cardiac edema disappears, the concentration of this ion falls.

Two other cases are reported in table 4 showing less striking changes with diuresis.

NEPHRITIC ACIDOSIS

Acidosis in nephritis was first observed by von Jaksch (15) in 1888. By titrating whole blood he discovered an "Alkalescenz" of 24 to 40 mgm. of NaOH per 100 cc. in uremia as compared to 250 to 300 mgm. in normals. Frequent confirmation since that date has left no doubt as to the relative accuracy of this early work. The first satisfactory hypothesis of the mechanism of nephritic acidosis was offered by Sellards (16) in 1914. He suggested that there is a "very gradual decrease in the excretion of acid in the urine and a corresponding reduction of carbonates in the blood corresponding to the amount of acid salts which are suppressed." At about the same time Palmer and Henderson (17) observed that a discrepancy in the usual relation between ammonia excretion and urinary acidity occurred in nephritic acidosis; this discrepancy lay in the relatively small amount of ammonia excreted as the urine became more acid. Since it has been shown that ammonia formation probably occurs in the kidney, their findings are easily explained, and impaired ammonia formation takes its place as one of the important factors in renal acidosis.

Sellard's hypothesis received further support when it was discovered that phosphate (Marriott (18)) and sulphate (Loeb and Benedict, 1927 (19)) retention occurred in uremia; the total electrolyte dis-

tribution, however, had not been investigated. Peters and his co-workers in 1923 did partial partitions of serum electrolytes in uremia and attributed acidosis to: (1) a reduction in total base, and (2) an increase in undetermined acids which he believed included a "marked increase in organic acids." The authors (1) in 1927 studied the effect of complete urinary retention in dogs by ligation of the ureters, demonstrating that in this simple experiment, phosphate and sulphate represent the entire increase in "undetermined acids" and easily account for the acidosis of this condition.

Hartmann and Darrow (20) in an excellent study in 1928 also observed low base levels in chronic nephritis. They explained this loss on the basis of (1) the work of Linder (*loc. cit.*) (12) and others *who have shown that the damaged kidney cannot normally conserve base by ammonia formation*; and (2) the loss of BCl due to the polyuria of advanced nephritis. The first suggestion seems to require no further support but the second, while attractive, remains to be proved experimentally. As a matter of fact it is the inability of the kidney to save base that makes the polyuria significant for it can be seen in the data presented under "diuresis" that large loss of body fluids through normal kidneys can occur with considerable decrease in serum chloride and yet no change in total base. Hartmann and Darrow believe that phosphate and sulphate account for the increase in undetermined acids. They found lactic acid to be unimportant except during convulsions, and ketones to be negligible.

More recently Peters, 1929, (4) reaffirmed his conclusions of 1923, offering the additional suggestion that ketosis due to starvation may be an important factor and further states (p. 529) that "sulphate accumulations must play a comparatively minor rôle in the production of the acidosis of nephritis." The dependence of phosphate and sulphate retention upon renal insufficiency could be proved only by successful studies of renal physiology, but the fact remains that many observers have found a fairly consistent relationship between severe kidney damage and phosphate and sulphate retention. At least one investigation (Boyd, *et al.* (21)) demonstrated increased blood phosphates in those cases where urine phosphate excretion was strikingly decreased.

As Peters and co-workers were unable to include sulphate and ke-

SERUM ELECTROLYTE STUDIES

TABLE 5
Nephritic acidosis

Date	Initials, hospital number and sex	Case number	Serum										Vomiting	Remarks
			Nonprotein nitrogen mgm. per 100 cc.	Serum protein m. Eq. per liter	Total base m. Eq. per liter	Total acid m. Eq. per liter	B - A m. Eq. per liter	Chloride m. Eq. per liter	Carbonate m. Eq. per liter	Phosphate m. Eq. per liter	Protein m. Eq. per liter	Sulphate m. Eq. per liter	Ketones m. Eq. per liter	
June 11, 1926	P. S. 56851 Male	1	160	7.1	142.5	139.0	3.5	88.5	20.6	5.4	16.8	7.7		± A rapidly fatal uremia in a man of 39, vomited 12 hours before blood taken. Autopsy: Chronic glomerular nephritis
March 20, 1929	B. 80150 Male	2	195	6.5	142.8	137.9	4.9	81.6	21.5	6.8	15.3	12.7	0	++ A man of 22 with repeated attacks of acute nephritis with 1 month vomiting and uremia
March 28, 1929	Mo. 80644 Male	3	231	5.7	146.7	144.7	2.0	105.2	12.4	8.7	13.5	4.8	0	0 0 ++ A typical nitrogen retention nephritis of 29, dying April 11, 1929. Autopsy: Chronic glomerular nephritis. Fluids forced between March 28 and April 3 with polyuria after that date persistent vomiting
April 3, 1929			229	6.1	141.6	135.8	5.8	89.8	11.2	12.2	14.4	8.2	0	
April 11, 1929			390	6.6	137.6	132.1	5.5	60.4	11.9	23.2	15.6	19.5	1.5	
March 29, 1929	D. G. 80898 Female	4	208	7.0	153.9	148.9	5.0	96.4	16.6	10.7	16.5	7.0	1.7	0 A rapidly fatal uremia in a girl of 20

March 13, 1929	M. A. 80139 Female	5	91	5.1	147.5	145.2	2.3	104.4	20.6	4.8	12.0	3.4	0	0	Undernourished woman of 33 with 4 years of edema—very severe secondary anemia—very little uremia
April 17, 1929	Bur. 80036 Female	6	107	7.0	144.2	141.3	2.9	97.8	19.9	4.6	16.5	2.5	0	++	Pregnant woman of 29 with 5 years of cardiac insufficiency, obesity, hypertension, becoming uremic and dying thus
July 21, 1928 July 27, 1928	Map. 69185 Male	7	173 160	5.6 5.7	145.2 146.3	136.1 131.8	9.1 14.5	76.4 63.8	30.2 36.9	10.4 12.0	13.2 13.5	5.9 5.6	++ ++	++ ++	See appendix. July 27, 1928, Creatinin, = 29.2 mgm.



FIG. 5—Case 80644

tone analyses, we wish to present a group of cases studied during the past three years in whom sulphates and ketones were determined. Table 5 presents ten observations on seven cases of terminal uremia.

As a group these electrolyte studies show general characteristics similar to those that have been discussed above. Total base is below normal in all but one instance, and it is not possible with our cases to correlate this reduction with the vomiting, for it occurred even in the absence of this complication. Nor is it coincident with loss of chloride, as is shown by cases 80644 and 80139 who had normal chlorides and low total bases. Again, bicarbonate loss is obviously not the determining factor as can be seen in case 69185. Comparing total base with chloride plus bicarbonate shows equally inconsistent relationships. Case 80644 illustrates very clearly the inability of certain uremic individuals to conserve base in the presence of polyuria. Between the first two observations, the fluid intake was high and the resulting polyuria was followed by a decrease in base of 5.1 m.Eq. and in chloride of 15.4 m.Eq. In spite of these changes and in the presence of excessive water elimination through the kidneys, phosphate and sulphate concentrations steadily increased. This observation is contrary to the results of Denis and Reed (22) who noted in nephritic dogs a rapid drop of sulphate levels during diuresis.

On the acid side the effects of uremia are more varied and complicated. In the first place, it is obvious that ketones are quantitatively of no significance, even in the presence of excessive vomiting; in the second place, the importance of phosphate and sulphate retention is equally clear. It would be futile to insist, from our data, that sulphate and phosphate retention is the primary mechanism. The picture is too complicated and the physiological background too obscure. However, in a similar situation, i.e., in dogs with ligated ureters, the authors (*loc. cit.*) have shown that sulphate and phosphate accumulate with no apparent channel for chloride loss. There was no urine, of course, and a negligible chloride content in the vomitus. It would seem that there, at least, was a simple displacement of bicarbonate and chloride by retained acids, a relationship that might be carried over by analogy to the present data. To be sure, in uremia other factors complicate the picture, as has already been noted.

The effect of vomiting on chloride loss is most apparent in cases

80644 and 61985, who, when vomiting excessively, showed the lowest chloride concentration of the series. In case 80644 this complication became so pronounced that true tetany with alkalosis occurred at a time when the creatinine was 29 mgm. per 100 cc. We have never, hitherto, observed tetany in uremic patients except after administration of bicarbonate, to which they react with unusual promptness. It is unfortunate that we did not investigate lactic acid and ketones in case 69185, for they might together have made up the uncommonly high "undetermined acids."

It can readily be seen that there is, in most instances, little discrepancy between total base and total acid when sulphate studies are included. $B - A$ is slightly higher in this group than in normals, but the assumed pH tends to be more incorrect and, therefore, the calculation of phosphate and protein is less certain, altogether making a limit of error close to the differences found. Even these differences might be cut down by the inclusion of lactate analyses. Hence the presence of other organic acid is not established by the data in table 5.

In summary, the essential disturbances of the electrolytes in uremia are: (1) decrease in total electrolytes; (2) decrease in total base due in part, at least, to defective base conservation by the kidney and, occasionally, to vomiting; (3) accumulation of phosphate and sulphate, due presumably to renal insufficiency; (4) decrease in chloride, related at times to vomiting, but still incompletely explained; (5) decrease in bicarbonate due to retention of inorganic acids (sulphate and phosphate).

DIABETIC ACIDOSIS

Peters and his associates in 1925, (23) published the first analysis of the factors producing serum electrolyte changes in diabetic acidosis. The validity of their hypotheses has received support by later and more complete analytical data. Peters called attention to: (1) loss of water, because of glycosuric polyuria, base excretion and vomiting; (2) loss of base bound to ketones and thus excreted in the urine; and (3) availability of base released from chloride for ketone neutralization. There results, therefrom, a state of dehydration and salt depletion, as well as acidosis.

Hartmann and Darrow, in 1928 (24), studied diabetic acidosis in

TABLE 6
Diabetic acidosis

Date	Hospital number and sex	Case number	Serum										Ketones	Blood sugar
			Non-protein nitrogen	Serum protein	Total base	Total acid	B - A	Chloride	Carbonate	Phosphate	Protein			
			mgm. per 100 cc.	per cent	m. Eq. per liter	m. Eq. per liter	m. Eq. per liter	m. Eq. per liter	m. Eq. per liter	m. Eq. per liter	m. Eq. per liter	m. Eq. per liter	m. Eq. per liter	mgm. per 100 cc.
April 22, 1926	54592	1	39	9.4	143.8	147.6	-3.8	97.4	9.6	3.2	22.2	15.2±		
April 23, 1926	Male		27	7.8	135.0	134.5	0.5	96.4	18.4	1.3	18.4	0		
March 30, 1926	62918	2	71	6.6	124.0	110.8	13.2	82.3	5.5	7.4	15.6			
June 1, 1926	Female													
	Female	3	50	6.0	141.9	129.7	12.2	100.4	13.6	1.5	14.2			
January 3, 1927	58570	4	51	7.3	139.3	127.7	11.6	105.2	3.6	1.7	17.2		5.40	
January 4, 1927	Female		24	5.7	148.8	142.3	6.5	102.8	26.0	0.0	13.5		0.46	
January 7, 1927			26	5.8	153.3(?)	143.4	9.9	102.8	26.0	0.9	13.7		1.52	
March 22, 1926	65330	5	87	8.7	114.0(?)	119.8	-5.8	93.0	5.2?	?	20.1	1.5		
March 24, 1926	Male		34	5.5	139.0	139.4	-0.4	105.4	18.3	0.6	13.0	2.1		
April 26, 1926			39	7.4	143.5	145.6	-2.1	97.6	28.1	2.4	17.5			
March 30, 1927	66489	6	46	7.8	136.1	132.8	3.3	97.4	17.0	0	18.4			
March 30, 1927	Female		(46)	7.3	137.8	134.7	3.1	94.0	23.5	0	17.2			
April 2, 1927			24	6.6	142.4	138.5	3.9	93.2	28.4	1.3	15.6			
April 7, 1927			24	7.5	145.0	140.3	4.7	96.2	24.2	2.2	17.7			
June 3, 1927	61945	7	39	5.1	128.8	123.2	5.6	92.0	17.1	2.1	12.0			
June 6, 1927	Male		27	5.9	148.1	145.7	2.4	99.2	30.9	1.7	13.9			
June 27, 1928	63959	8	49+	8.5	141.5	140.1	1.4	90.8	4.9	4.1	20.0	20.3±		
June 28, 1928	Female		28	6.9	148.6	149.1	-0.5	102.6	28.7	1.5	16.3			
September 27, 1928	74710	9	64	7.5	138.9	132.8	6.1	87.8	5.9	4.1	17.7	17.3±		
September 28, 1928	Female		52	6.6	159.8	153.6	6.2	110.8	26.4	0.8	15.6			

children, and observed dehydration, loss of base and chloride. Most of their observations do not include complete electrolyte partitions.

In a group of 9 cases, we have been able to make more comprehensive analyses than hitherto reported. The determinations are found in table 6 and the case summaries are reported in the Appendix. In four cases ketones were directly measured but in the others they are found as part of B - A. Certain aspects peculiar to individual cases should be mentioned before engaging in general discussion.

The episode in case 54592 was extremely simple; no acute infection, very little vomiting and prompt recovery. At the end of the period there was no dehydration and the blood contained no ketones. The fact that the blood ketone figure was high enough to cause a negative B - A indicates clearly that it should be corrected downward at least 4.0 m.Eq., for we have already pointed out the inaccuracy of the

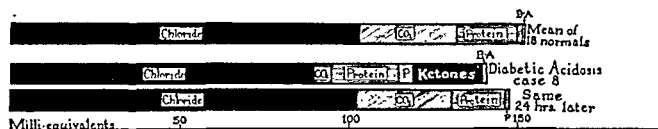


FIG. 6—Case 63959

calculation of m.Eq. from grams per liter of ketones. However, assuming 11.0 m.Eq. of ketones, there is a total anion drop in the 24 hours of 17.7 m.Eq. with only 8.8 m.Eq. of the liberated base forming bicarbonate; there was, therefore, a large loss of fixed base (8.8 m.Eq. as determined). There was no vomiting to explain this loss. Hence, it seems probable that the decrease in serum base represents base bound to ketones and excreted in the urine. Diuresis with extensive urinary elimination of ketones did occur. From the serum studies alone one can suggest that the disappearance of ketones was due, in part, to oxidation and, in part, to excretion. This patient obviously had too little NaCl in the course of treatment.

Case 62918 shows the extraordinary degree to which decrease of base, chloride and bicarbonate can occur with dehydration, the latter being indicated by a high protein per cent. Persistent vomiting played a major rôle here in the loss of BCl.

Case 65967 requires no special comment other than to note the effect of treatment in maintaining satisfactory levels of base and chloride. She died of shock. In both this case and in case 62918 the striking increase in $B - A$ probably indicates the extent of the ketonemia.

No case except 58570 had a normal chloride at the onset; in this patient it was slightly above normal. As far as we know there had been no therapy that would explain this. After vigorous treatment the blood returned to a fairly normal level with a drop in Cl . The value for total base 3 days later was so out of line with the constant results for the other electrolytes that there appeared to have been an error in the measurement.

Case 66489 requires little comment except to observe that the administration of bicarbonate between the first and second samples increased the bicarbonate to some extent at the expense of chloride.

The low base concentration after treatment and after a very brief period of vomiting is noteworthy in Case 61945. This boy vomited only 12 hours and was then given vigorous and proper treatment with clinical recovery at a time when his total base was 128.8 m.Eq., although his blood contained practically no ketones.

Case 63959 was severely dehydrated with very low bicarbonate and chloride and high blood ketones. Base, however, in spite of these extensive alterations was less reduced than usual. Twenty-four hours of energetic treatment removed ketones and readjusted the electrolytes to a normal pattern.

Case 65330 illustrates the importance of shock in diabetic acidosis and its independence of ketones. This boy was near death from vasomotor collapse at a time when there was a relatively low concentration of ketones in the blood. About 24 hours later he grew worse again with a blood CO_2 that was not in the dangerous zone. Tremendous dehydration and salt depletion are apparent in the first analyses, inaccurate though they may be. (See appendix.)

Strenuous treatment in case 74710 resulted in overreaching the electrolyte needs, as shown by an abnormally high base and chloride in the last study. This treatment was necessary in order to combat shock, which was a very serious complication here. It shows, however, that it is possible correctly to readjust the electrolyte pattern by means of active therapy.

The mechanism of diabetic acidosis probably proceeds somewhat as follows. First, there is a production of ketone acids in a patient with already low electrolyte levels (due to decreased base and chloride). These acids take base from bicarbonate with loss of CO_2 through the lungs. At the same time, ammonia formation in the kidneys furnishes base for the elimination of ketones in the urine, although evidence increasingly emphasizes the ready availability of fixed base, even before ammonia. Increased water flow through the kidneys due to glycosuria may be of some assistance in the elimination of ketones at this stage.

Large quantities of ketones with fixed base as the cation appear in the urine, and salt depletion is further enhanced by the almost simultaneous appearance of vomiting which means loss of base, chloride and water. The final effect is a dehydrated blood, low in total base, chloride and bicarbonate, and high in ketones.

The dehydration causes a drop in blood volume with consequent decrease in blood pressure. Whether ketones themselves are vascular poisons and contribute thus to shock is not certain. At any rate vasomotor collapse appears regularly. The fall in blood pressure plus the increased osmotic pressure of the dehydrated serum (due to the rise in protein per cent) interferes greatly with renal function as the consistently increased nonprotein nitrogen seems to confirm. When anuria occurs one of the most important protective mechanisms fails so that the disturbances are even more pronounced and a vicious circle is initiated.

Retracing these steps therapeutically, the indications are: (1) restoration of blood volume and normal dilution by salt solution, if possible, and transfusion, if necessary (but by the intravenous route exclusively until blood pressure is normal); (2) replacement of base, chloride and, to a lesser degree, bicarbonate by intravenous introduction; and (3) interruption of ketone formation by glucose and insulin administration.

GENERAL DISCUSSION

The accumulation of data concerning serum electrolytes has reached a stage where certain simple generalizations are justified.

In the first place, the total concentration of ions is far less constant

than some earlier writers believed. Under a variety of conditions the levels are greatly lowered, (15 to 30 m.Eq.) but very rarely indeed in disease is there an elevation. Work by Bock (25) on fatigue and work from this laboratory (in press) on histamine shock demonstrate increase in serum fixed base as part of the response to lactic acid acidosis. Apparently the electrolyte concentration does not determine the water content of the serum, for within the relatively wide limits compatible with cell survival, water and electrolytes vary independently as various influences bear upon them. It is interesting that clinical recovery does not necessarily parallel the return of the electrolytes to normal.

Total base is very constant in casual studies of normals, but there are no satisfactory data as to the response to variations in diet or fluid intake. Most disease conditions studied to date cause a decrease in base if they influence it at all. This decrease is ordinarily dependent on cation loss in vomitus or urine. One is increasingly impressed by the ease with which fixed base is mobilized for urinary secretion under acid stress. In fact, some work suggests that it is a more mobile agent than ammonia formation (Odin, (26) and unpublished work of the authors). Base loss in diarrhea has not been included in this series but is well established as part of the pathological physiology of the cations. Although hypotheses as to the usefulness of fixed base in acidosis have become certainties, the complete mystery of the base changes in pneumonia indicates one of the many opportunities for further work in this field.

It seems clear that normal serum has no quantitatively significant acids other than chlorides, bicarbonate, phosphate and protein. Organic acids and sulphates are rarely more than 1 or 2 milliequivalents. It seems equally certain that in the disease conditions hitherto subjected to this type of scrutiny no discrepancy between total base and total acid has been found when sulphate, ketone and lactate analyses were included. The importance of complete electrolyte partitions consistently performed is apparent. Conclusions independent of such completeness are subject to errors even greater than those indigenous to all biological research.

When base is needed to neutralize acids pathologically present, bicarbonate offers it most readily but chloride takes an important part

when the load becomes heavy. It may even give up more base than does bicarbonate. There is an inconstant reciprocal relationship between chloride and bicarbonate; occasionally when chloride decreases, as in diuresis, bicarbonate fills the gap, but the reverse has not been observed, due probably to the lack of an analogous drop in bicarbonate.

The mechanisms which produce the changes in serum electrolytes found in diabetic and nephritic acidosis are relatively well understood and bear a consistent relationship to one another. Much has been accomplished there. But an understanding of the relation of edema and water balance to the electrolyte metabolism or an interpretation of the changes found in nephrosis, for example, are impossible with the evidence at hand.

SUMMARY

Serum electrolyte partitions have been made in normals, in pneumonia, during cardiac diuresis, in nephritic edema (nephrosis) and in nephritic and diabetic acidosis. The accuracy and reliability of the various methods have been discussed. The so-called "undetermined acids" sink to negligible figures if bicarbonate, chloride, protein, phosphate, sulphate and ketones are estimated in these conditions.

The mean total base of 18 determinations on *normals* is 151.9 m.Eq. and the total acid 151.7 m.Eq. Organic acids and sulphates are present in such small quantities as to be entirely unimportant.

In *pneumonia* there is a decrease in total base, chloride, phosphate and protein with a very slow and roughly parallel return to normal after the crisis. These changes received no elucidation from our data. There is no increase in undetermined acid.

Patients with *renal edema* showed invariably a low protein per cent and usually a low fixed base. Serum chlorides were often very high and increased as the result of chloride administration more strikingly than normals. A partial explanation of the behavior of the chlorides is offered by citing the effect of a decreased serum protein upon the Donnan equilibrium between serum and the fluids in tissue spaces.

Studies of *diuresis* in cardiac patients demonstrated the ability of the body to eliminate large amounts of chloride and water without parallel decrease in total base.

The *acidosis* of *advanced nephritis* was followed in several cases and the factors chiefly responsible seem to be: (1) decrease in total base dependent on loss of the base-conserving power of the kidney due to failure of ammonia formation; and (2) retention of phosphate and sulphate because of renal insufficiency. No significant ketosis was observed. BCl is a valuable source of base for neutralizing retained acids.

Observations on a group of patients suffering from severe *diabetic acidosis* showed: (1) dehydration with high serum protein; (2) loss of base, due probably to urinary excretion in combination with ketones; (3) great drop in bicarbonate dependent on neutralization of ketones; and (4) decrease in serum chlorides dependent in part on vomiting, but nevertheless a real aid in furnishing base for ketones. Shock plays an extraordinarily important rôle in treatment and prognosis. A return of total base to normal is not necessarily parallel to the clinical recovery.

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APPENDIX

Case 54592 was a man of 32, with diabetes of four years' duration. He was on a self-regulated dose of 60 units of insulin, but because of 2 severe shocks suddenly stopped all insulin. After one day of eating, without insulin, he began to vomit and was admitted at once to the hospital on the morning of April 22. He was dyspneic but not in coma. Blood was removed before any treatment. Between the initial sample and the second one, 20 hours later, he did not vomit and received 3000 cc. of fluids and 4 grams NaCl. During the same period he had a striking diuresis. Urine was free from ketones at the end of the period.

Case 62918 was a woman who had had diabetes for about 2 years. She was followed in the Out-Patient Department on a diet of C. 250; P. 70; F. 80 with 77 units of insulin. She had disappeared from the Clinic for 5 months before admission. On March 20 she had "grippe" mildly; on the evening of March 27 she began to vomit, which continued to admission; on March 30 she became hyperpneic and drowsy and was admitted to the hospital. Her "acidosis chart" was lost from the history so accurate data are unavailable. Her blood pressure dropped and she died in 10 hours

Case 65967 was a woman of 68 who had had diabetes for 12 years treated with casual dietary restriction. For 4 months there had been weakness and dyspnea on exertion, treated with digitalis by her local physician. For 2 weeks before admission she had been eating unusual quantities of rye bread and cream. About 12 hours before admission, on May 31st, she developed abdominal pain and vomiting, the latter continuing to her hospital entry. Her condition was poor on admission and her blood pressure continued dropping until death, at 9:20 p.m. June 1, 3 hours after the sample of blood was obtained. She received 3500 cc. of salt solution and 2500 cc. of 5 per cent glucose solution by vein or clysis in the approximately 36 hours between admission and removal of the blood sample. Cardiac failure may have played a rôle in her death, although it was due largely to shock.

Case 58570 was a woman of 40 who had had diabetes for 4 years, with varying tolerance but good general health. Four days before admission she caught cold,

lost her appetite and ate nothing for three days, taking no insulin. The day before admission she vomited and became gradually worse, arriving at the hospital January 1st in coma. Blood was taken before any therapy. She responded quickly to treatment with insulin, bicarbonate, salt solution and glucose, by clysis, vein and mouth. Her blood pressure was normal. When the second specimen was removed 23 hours later she was conscious, and voiding well and her urine was practically free from ketones. She was edematous at that time; on January 7 she was quite normal.

Case 65330 was a diabetic boy of 16 who had been progressing satisfactorily for three years on diet and insulin. On March 18 he had an upper respiratory infection; on March 20th he stopped insulin and began to vomit. He had diplopia, was thirsty and drowsy. On the 21st he was worse and entered the hospital at 4 p.m., pale, dehydrated, and hyperpneic. He was given insulin, and glucose and water by mouth and rectum. He improved at once but soon vomited again and became worse. His stomach was lavaged and 10 per cent glucose was given by clysis twice during the night. He became steadily worse, continued to vomit and early in the morning of March 22nd his blood pressure was 70/40; he was anuric and in shock. 500 cc. of normal saline and coffee by rectum helped but little. At noon a transfusion of 300 cc. of blood was given (the first sample in table 6 was taken just before it). In an hour the blood pressure was 90/60 and 120 cc. of urine was obtained which, on analysis, showed much albumin but very little of ketones. Salt solution by vein and rectum was continued and the boy improved until the afternoon of the following day, March 23, when he vomited, his blood pressure fell to 86 (blood CO_2 was 30.9 volumes per cent) and he grew drowsy. Intravenous saline brought his blood pressure up again and his improvement was steady thereafter. The second blood was taken after he was much improved. The third specimen was during a period of entire normality (except his diabetes) a month later. The data obtained on March 22nd are probably rather inaccurate due to poor technique in removing blood. The patient was so critically ill that it was impossible to prevent exposure to the air and a very small amount was obtained, quite insufficient for the usual duplicates.

Case 66489 was a diabetic girl of 22 who had been under treatment for 8 months. For six weeks she had been substituting a patent medicine for part or all of her insulin. Three days before admission she felt ill and took a purge; the following day she began to vomit. The next day she went into coma (no other details are available), and in the evening arrived at the hospital completely unconscious, hyperpneic, but not vomiting. Between admission and her first blood sample she received about 3700 cc. of fluids by mouth, 1000 cc. of 5 per cent glucose and 500 cc. of saline by vein. Between the first and second samples 2 hours elapsed during which 15 grams of bicarbonate were given intravenously. No ketones were excreted during that time. The third and fourth determinations were done at a

time when the patient had developed bronchopneumonia and pyelitis. Later, abscess of the lung and suppurative pleurisy caused her death on April 30.

Case 61945 was a 14 year old boy satisfactorily treated for diabetes over a period of three years. His diet was C. 200 P. 80 F. 130 and insulin 80 units. The day of admission (June 2) he awoke vomiting and continued all day without insulin or food. At 8 p.m. his CO_2 was 23 volumes per cent. He was given intravenous bicarbonate and saline, with the usual insulin and glucose. When blood was taken the next morning he was decidedly better and on June 6 he was quite well except for some glycosuria.

Case 63959 was a girl of 16 who had been successfully treated (with insulin) over a period of 3 years on a diet of C. 160 P. 65 F. 100. The day before admission she began to vomit, insulin was decreased and by afternoon of the day of admission (June 27) she was in coma. Blood was taken before therapy was started. Between the initial and final observations she had 1500 cc. of saline and 1500 cc. of 5 per cent glucose intravenously, 1000 cc. of saline subcutaneously and 1100 cc. of fluid by mouth; she was symptom-free and the urine was negative for ketones at the second study. Her blood pressure was always normal.

Case 74710 was a woman of 48 who came into the hospital late on September 26 for vomiting and was found to have diabetes; she went rapidly into coma and shock (blood pressure 88/58). There was no apparent precipitating factor. Blood was taken before treatment. Between the first and second samples she received approximately 800 cc. of whole blood and 3000 cc. of fluid intravenously, including 20 grams of bicarbonate and 36.4 grams of NaCl. She was much improved but the urine still showed large amounts of ketones.

Case 69185 was a woman of 38 with a history of toxemia of pregnancy. Fourteen years previously she had had headaches, ankle edema and dyspnea on exertion for 8 months with, more recently, blurring of vision, drowsiness and vomiting. Blood pressure 175/95, heavy albuminuria, normal fundi, oliguria persistently (250-500 cc.). She vomited large amounts constantly. She received infusions as follows:

July 2, 1928, 1000 cc. of 10 per cent glucose.

July 3, 1928, 1000 cc. of 5 per cent glucose and normal saline.

July 4, 1928, 1000 cc. of 5 per cent glucose and normal saline.

July 11, 1928, 1000 cc. of 10 per cent glucose.

July 12, 1928, 500 cc. of 10 per cent glucose.

July 23, 1928, 400 cc. of 10 per cent glucose and 200 cc. normal saline.

July 24, 1928, 500 cc. of 10 per cent glucose.

July 27, 1928, 1000 cc. of normal salt and 30 cc. of 50 per cent glucose.

She developed tetany on July 29 and died in coma on July 31.

THE COMPOSITION OF THE BILE FOLLOWING THE RELIEF OF BILIARY OBSTRUCTION¹

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The significance of the liver, as the largest glandular organ in the body, and its paramount importance in certain phases of intermediary metabolism, contrasts strangely with the paucity of knowledge, at least in man, concerning its secretion, the bile. The characteristic biliary constituents, bilirubin, the bile acids and cholesterol have been isolated and identified chemically. Experimental work on animals, especially on dogs with permanent biliary fistulas, has indicated the multiplicity of factors, such as diet, climate, fasting, various chemical poisons, infections and exercise, which may affect either the volume or composition of the bile. In this connection, the earlier work of Bidder and Schmidt (1852), and of Stadelmann (1891, 1896) and, in recent years, that of Whipple (1922) and Rous (1925) and their collaborators has been of fundamental significance.

As a result of these studies it is now recognized that there may be great variations in the flow of bile from day to day. The secretion of bile pigment, on the other hand, continues at a fairly constant rate except for changes due to hemorrhage, anemia or other disturbances in the metabolism of the blood pigments. In consequence, the concentrations of the bile pigments tends to vary inversely with the volume of the bile, and McMaster, Broun and Rous (1923), and Greene and Snell (1928) have emphasized the extreme variations in concentration which occasionally may be found. The intimate relation between the diet and the excretion of cholesterol in the bile has recently been

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commented on by McNee (1914) and by McMaster (1924). Several factors combine to determine the excretion of bile acids: (1) an endogenous factor responsible for the relatively constant excretion of bile acids in the fasting dog which may possibly be related to the metabolism of body protein; (2) an exogenous factor, determined by the diet and especially concerned with the amount and character of the protein therein, and (3) an intestinal factor as a result of the absorption of bile acids from the bowel, with the resultant establishment of the enterohepatic circulation originally postulated by Schiff (1870); this intestinal factor is further modified as a result of changes in the intestinal flora and variations in the destruction of bile acids by bacterial action. The functional state of the hepatic cells must also be taken into consideration, for changes in the functional efficiency of the liver as a result of the production of an Eck fistula, cholangitis, or the administration of hepatic toxins, such as chloroform or phosphorus have a profound influence on the excretion of the bile acids. Greene and Snell (1928) found the variation in the attainable concentration of bile acids in the bile to be relatively slight, at least when compared to the observed variations in the concentration of bilirubin. Under some conditions, therefore, the volume of the biliary output may be determined by the excretion of the bile acids, although this relation does not always hold and the two may be entirely independent the one of the other.

Direct evidence regarding the secretion of bile in normal men, and the modifications accompanying disease, is lacking. The greater part of the available data is concerned with the composition of bile from the gallbladder but because of the concentrating action of that viscus such analyses do not indicate the composition of the bile as initially formed in the liver. Similar criticisms apply to the studies of Rosenthal and von Falkenhausen (1923), and of McClure (1925) and his associates, who used the material obtained by duodenal drainage. Apart from the uncertainty, as to how far the duodenal content is representative of the hepatic secretion, there is further difficulty in that only the concentration, and not the total amount of the different biliary constituents, is determinable. Numerous studies have been made of bile obtained from patients with permanent biliary fistulas. Unfortunately, the majority of such investigations was made before

the development of adequate analytic methods and so were concerned chiefly with the changes in the volume and total solids present in the bile.

More recently von Czyhlarz, Fuchs and von Fürth (1913), Chabrol, Bénard and Bariéty (1926), and Rosenthal, von Falkenhausen and Freund (1926) have reported single cases or small groups of cases in which the composition of the bile has been carefully studied during the first few days after cholecystectomy and drainage of the common bile duct. We wish to report the changes in an additional series of nine cases. Such cases afford an opportunity to study the effect of biliary obstruction on the secretion of bile and the return toward normal following relief of the obstruction. It is possible, in consequence, to compare the changes observed clinically in patients with those in dogs reported by McMaster, Broun and Rous (1923) following temporary occlusion of a permanent biliary fistula. Furthermore, the differences in the response of the different cases afford additional light on the disturbances in the functional activity of the liver as a result of biliary obstruction or infection.

DETAILS OF INVESTIGATION

The drainage of bile from the gallbladder or common bile duct, subsequent to the relief of biliary obstruction, was studied in nine cases. Details regarding the clinical histories of certain of these cases are given elsewhere (26). The methods of analysis were those used in previous studies of this series: the method of Greene, Snell and Walters (1925) for bilirubin; of Aldrich and Bledsoe (1928) for bile acids; of Van Slyke (10) for chlorides, and of Van Slyke and Cullen (10) for urea. The data obtained in the different cases are given in the accompanying tables. It is recognized that the collection of bile from a drainage tube inserted in the gallbladder or common bile duct is not always complete. In the present series the analysis of bile was continued during the whole period of observation but the tables include only the data obtained during the period when the collection was apparently complete. For purposes of study the cases have been divided into three groups.

Group 1. Tables 1, 2 and 3, and figure 1A comprise three cases of chronic cholecystitis with stone in the common bile duct. In these

cases, the histories were of typical, intermittent biliary obstruction, with mild degrees of jaundice. The liver was apparently normal at the time of operation. Cholecystectomy, choledocholithotomy and choledochostomy were performed in all, and the postoperative course was uneventful.

Group 2. Tables 4, 5 and 6 and figure 1*B* contain three cases. One patient had chronic cholangitis, biliary obstruction and a consequent obstructive type of biliary cirrhosis; a T-tube was inserted in the common bile duct. Another patient had chronic cholecystitis with stones, a stone in the common bile duct, and considerable biliary cirrhosis; a T-tube was inserted in the common bile duct after removal of the stone. The third patient had complete biliary obstruction due to carcinoma of the head of the pancreas, but also had cirrhotic changes in the liver in consequence of the obstruction; cholecystostomy, as a preliminary to cholecystenterostomy, was performed.

Group 3. Increase in the biliary drainage, with paling and thinning of the bile, is one of the symptoms of postoperative hepatic insufficiency in cases of obstructive jaundice. Walters and Parham (1922) have pointed out the serious prognostic importance of cholerrhagia. This condition was present in the three cases in group 3 (tables 7, 8 and 9 and figures 1*C* and *D*). The patients gave a history of marked and painless jaundice of from two to six months' duration. In two cases there was a carcinoma of the head of the pancreas and cholecystostomy was done, preliminary to cholecystenterostomy. In the third patient there was a carcinoma of the common and hepatic bile ducts, and only choledochostomy could be done.

COMMENT

McMaster, Broun and Rous (1923) have described in detail the changes, in dogs, produced in the character of the bile as a result of obstruction to a permanent biliary fistula and the response to the relief of such an obstruction. They pointed out that with partial biliary obstruction there was a reduction in the output of pigments, of bile acids and of cholesterol, and to a much greater degree than that in the volume of bile, so that the fluid elaborated by the liver became progressively poorer in the typical biliary constituents. Because of the analogy with changes in the renal secretion incident to the develop-

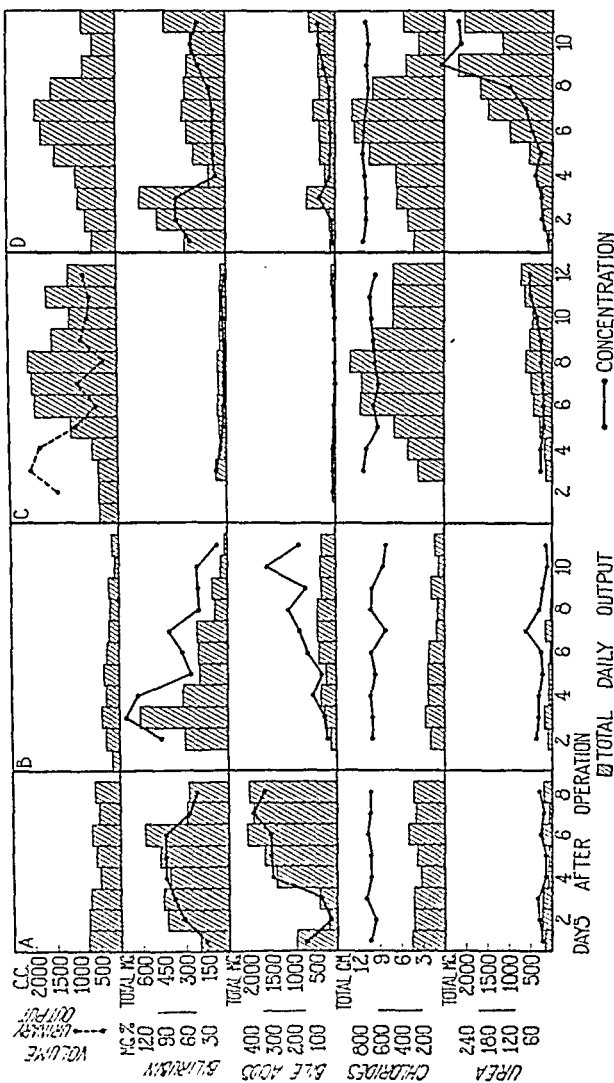


FIG. 1. THE COMPOSITION OF THE BILE FOLLOWING THE RELIEF OF BILIARY OBSTRUCTION

A, case of chronic cholecystitis with stone, illustrating group 1; B, case of chronic cholangitis with obstructive biliary cirrhosis, illustrating group 2; C, case of carcinoma of the pancreas with obstructive jaundice, illustrating cholangitis; the urinary output is shown; D, carcinoma of the common bile duct with obstructive jaundice, illustrating group 3 and the development of cholangitis and uremia.

ment of hydronephrosis, they used the term hydrohepatosis to designate the changes in the liver. They also pointed out that in the presence of complete biliary obstruction, the concentrating activity of the gallbladder may mask these changes either in whole or in part. Following relief of the biliary obstruction, a copious amount of bile was elaborated by the liver and the flow persisted until the greater part of the retained biliary constituents had been eliminated. This bile was more dilute than normal although the increase in volume was

TABLE 1
Summary of data in case 1 (group 1)

Days after operation	Bile									Blood serum*		
	Volume	Bilirubin		Bile acids as glycocholic acid		Urea		Chlorides as sodium chloride		Bilirubin	Urea	Sodium chloride
		Concentration	Total	Concentration	Total	Concentration	Total	Concentration	Total			
		mgm. per 100 cc.	mgm.	mgm. per 100 cc.	mgm.	mgm. per 100 cc.	mgm.	mgm. per 100 cc.	grams			
1	650	31	200	145	940	25	162	693	4.50			
2	655	64	425	26	170	36	236	645	4.23			
3	600	75	450	70	420	39	234	723	4.35			
4	475	87	414	296	1,410	18	85	681	3.24		38	585
5	550	87	478	308	1,690	18	99	681	3.75			
6	675	87	587	308	2,080	32	216	708	4.77			
7	500	56	280	387	1,930	24	120	692	3.46			
8	600	45	270	339	2,040	36	216	687	4.13			

* Before operation the following values in milligrams in each 100 cc. were determined: serum bilirubin, 2.8; blood urea, 24; serum chlorides as sodium chloride, 613.

such that the total output of pigment was increased during the period of choleresis.

Similar changes are found in many cases following the relief of biliary obstruction. The initial changes depend on the character of the bile present in the biliary tract at the time of operation. If it is thick and viscid as a result of the concentrating action of the gallbladder, there is an initial decrease in the concentration of the biliary drainage as this retained "stasis" bile is washed out (tables 1 and 2). If, on the other hand, "white" bile is present in the bile ducts, the fluid initially obtained may contain only minimal quantities of bili-

TABLE 2
Summary of data in case 2 (group 1)

Days after operation	Bile									Blood serum*		
	Vol- ume	Bilirubin		Bile acids as glycocholic acid		Urea		Chlorides as sodium chloride				
		Concen- tration	Total	Concen- tration	Total	Concen- tration	Total	Concen- tration	Total			
		cc.	mgm. per 100 cc.	mgm.	mgm. per 100 cc.	mgm.	mgm. per 100 cc.	mgm.	mgm. per 100 cc.	grams	mgm. per 100 cc.	mgm. per 100 cc.
1	130											
2	190	128	244	138	262	50	95	689	1.31	1.3	25	511
3	250	97	243	134	335	37	92	617	1.54			
4	175	71	124	333	583	24	41	675	1.18			
5	215	51	110	397	855	20	43	675	1.45			
6	200	77	154	513	1,026	13	26	648	1.29			
7	175	101	177	545	955	21	37	611	1.07			
8	150	112	168	450	675	16	24	619	0.93			
9	150	103	155	800	1,200	13	20	628	0.94		24	549
10	145	62	90	1,064	1,540	8	12	539	0.78			

* Before operation the following values in milligrams in each 100 cc. were determined: serum bilirubin, 1.5; blood urea, 25; serum chlorides as sodium chloride, 519.

TABLE 3
Summary of data in case 3 (group 1)

Days after operation	Bile									Blood serum*		
	Volume	Bilirubin		Bile acids as glycocholic acid		Urea		Chlorides as sodium chloride				
		Concen- tration	Total	Concen- tration	Total	Concen- tration	Total	Concen- tration	Total	Bili- rubin	Urea	Sodium chloride
		cc.	mgm. per 100 cc.	mgm.	mgm. per 100 cc.	mgm.	mgm. per 100 cc.	mgm.	mgm. per 100 cc.	grams	mgm. per 100 cc.	mgm. per 100 cc.
1	200											
2	350											
3	365	44	160	66	240	50	183	619	2.26			
4	700	19	133	62	434	55	385	460	3.32	3.6	24	507
5	600	79	474	387	2,320	92	550	723	4.34			

* Before operation the following values in milligrams in each 100 cc. were determined: serum bilirubin, 3.8; blood urea, 15; serum chlorides as sodium chloride, 1.

rubin or bile acids. The changes reported by Rosenthal, von Falkenhäusen and Freund (1926), and by Chabrol, Bénard and Bariéty (1926)

response when the obstruction to the biliary passages had not been sufficiently long continued or complete to produce permanent hepatic injury. Smyth and Whipple (1924) pointed out that in dogs a reduction in the excretion of bile acids is produced by a dose of chloroform so small as to be incapable of causing any recognizable structural change in the hepatic epithelium. Similarly, reduction or cessation in the production of bile acids would seem to be a characteristic effect

TABLE 7
Summary of data in case 7 (group 3)

Days after operation	Urine in twenty-four hours*	Bile									Blood serum*		
		Volume	Bilirubin		Bile acids as glycocholic acid		Urea		Chlorides as sodium chloride				
			Con- centra- tion	Total	Con- centra- tion	Total	Con- centra- tion	Total	Con- centra- tion	Total	Bili- rubin	Urea	Sod- ium chloride
			cc.	cc.	mgm. per 100 cc.	mgm.	mgm. per 100 cc.	mgm.	mgm. per 100 cc.	mgm.	mgm. per 100 cc.	mgm.	mgm. per 100 cc.
1		450											
2	1,425	475			13	62							
3	2,025	480	16	77	17	82	35	168	752	3.62	16.5	41	
4	1,800	650	9	58	11	71	35	227	711	4.62			519
5	1,000	1,100	5	55	4	44	25	275	609	6.70			
6	500	1,825	4	73	3	55	22	402	649	11.84			
7	950	1,950	3	59	1	19	26	506	601	11.70	13.5		
8	350	2,050	3	61	2	41	31	635	637	13.05			
9	850	1,550	3	46	1	16	33	512	641	9.95			
10	700	1,100	3	33	1	11	45	495	667	7.34	13.7	33	
11	650	1,650	4	46	6	69	60	690	685	7.87			
12	800	1,240	3	37	7	87	63	781	633	7.85	14.3	38	639

* Before operation the following values were determined: urine, cc. in 24 hours, 606; serum bilirubin, mgm. in each 100 cc., 25; serum urea, mgm. in each 100 cc., 15.

of biliary obstruction, and confirmation of this view is furnished by the experiments of Brakefield and Schmidt (1926) and of Snell, Greene and Rowntree (1927). If the injury to the liver in consequence of the obstruction is not too great, recovery is rapid and the concentration and total amount of bile acids in the bile rapidly return toward normal. In no case was there evidence for the washing out of any appreciable quantity of retained bile acids.

Hepatic injury and the development of obstructive biliary cirrhosis, as in the cases in group 2, modified the response of the liver to the relief of the obstruction (tables 4, 5 and 6, and figure 1B). The changes in the curve of excretion of bilirubin were not significantly different from those in the preceding group of cases although possibly the values were reduced slightly. Both the concentration and the total output of bile acids, on the other hand, were reduced and remained low throughout the period of observation. We believe this

TABLE 8
Summary of data in case 8 (group 3)

Days after operation	Bile									Blood serum*		
	Volume	Bilirubin		Bile acids as glycocholic acid		Urea		Chlorides as sodium chloride		Bilirubin	Urea	Sodium chloride
		Concentration	Total	Concentration	Total	Concentration	Total	Concentration	Total			
		mgm. per 100 cc.	mgm.	mgm. per 100 cc.	mgm.	mgm. per 100 cc.	mgm.	mgm. per 100 cc.	mgm.			
1	1,935											
2	3,025											
3	1,635	9	155			21	330			7.5	28	
4	1,540	10	155			21	320					
5	1,530	10	153	15	237	17	260	540	8.31	7.2	17	565
6	1,750	9	161	16	284	17	300	540	9.51	7.4	17	521
7	2,000	12	248	14	274	17	340	520	10.4	7.6	19	637
8	2,700	7	156	21	556	16	330	600	16.2	8.8	20	599
9	1,850	7	122	14	257	25	460	560	10.2	8.8	21	
10	2,800	6	162	20	550	26	730	560	15.6	9.2	32	563
11	2,400	6	130	7	350			580	12.2	9.8		

* Before operation the following values in milligrams in each 100 cc. were determined: serum bilirubin, 14.2; blood urea, 13.

indicates failure of recuperation by the liver and so is further evidence of the functional disturbance produced by the combined effect of the biliary obstruction and infection present in these cases.

In the cases in which marked cholerrhagia developed, the changes in the composition of the bile were most marked (tables 7, 8 and 9, and figure 1C and D). This supports the clinical impression that cholerrhagia is a symptom of serious hepatic dysfunction. In this condition the concentration and total output of bilirubin are somewhat reduced,

whereas the reduction in the bile acids is even more striking. Surgeons (Judd and Lyons, 1923) have recognized that the presence of white bile in the biliary tract ordinarily is evidence of the accumulation within the gallbladder and biliary passages of mucus and of the secretion of the mucous membrane lining these ducts, and this conception has been amply confirmed by the experimental studies of Rous and McMaster (1921). A second variety of white bile has been recognized, on the other hand, as occasionally appearing after the

TABLE 9
Summary of data in case 9 (group 3)

Days after operation	Bile									Blood serum*		
	Volume	Bilirubin		Bile acids as glycocholic acid		Urea		Chlorides as sodium chloride				
		Concentration	Total	Concentration	Total	Concentration	Total	Concentration	Total	Bili- rubin	Urea	Sodium chloride
		cc.	mgm. per 100 cc.	mgm.	mgm. per 100 cc.	mgm.	mgm. per 100 cc.	mgm.	mgm. per 100 cc.			
1	550	50	292	14	77	15	82	757	4.17	7.0	37	589
2	700	68	477	17	119	33	231	703	4.92			
3	875	68	595	71	622	30	263	707	6.20			
4	900	14	126	23	207	42	379	723	6.50			
5	1,400	16	224	22	308	39	546	740	10.35			
6	1,700	16	274	19	323	56	952	730	12.40	5.0		
7	1,825	16	292	27	493	80	1,460	707	12.90			
8	1,450	19	275	23	334	119	1,725	675	9.80			
9	710	36	256	50	355	304	2,160	703	5.00			
10	500	45	225	69	350	254	1,270	681	3.40			
11	760	36	374	71	540	263	2,000	707	5.38	178	509	

* Before operation the following values in milligrams in each 100 cc. were determined: serum bilirubin, 12.9; blood urea, 16.

institution of biliary drainage, and the data in case 7 illustrate the development of the latter condition. In this case the fluid secreted by the liver was almost completely acholic. Bile pigments were not present in as great a concentration as in the blood serum, whereas the sensitive Pettenkofer test showed the presence of but traces of bile acids. The characteristic constituents of the bile were entirely lacking, to all practical purposes, even though this deficient secretion was elaborated in considerable quantity. In this case, the development of

hydrohepatosis had apparently progressed to a stage of almost complete abolition of the biliary functions of the liver.

In all of these cases the concentration of urea in the bile was approximately equal to that in the blood, and the daily output was, therefore, determined by the volume of the bile. In one patient (case 9, figure 1*D*) postoperative renal insufficiency developed, and the concentration of urea in the blood increased to 300 mgm. in each 100 cc. The concentration of urea in the bile was increased in a similar manner. Marked cholerrhagia developed in this case at about the same time, with the result that the output of urea was increased to approximately 2 grams a day. Such an elimination of urea in the bile ordinarily would not be significant from the standpoint of renal function because the urea would be promptly reabsorbed on the passage of the bile into the intestine. In this case, however, the external drainage of the bile permitted vicarious elimination of the urea and consequent reduction in the burden borne by the kidneys.

Foster, Hooper and Whipple (1919) reported the development of abnormalities in the bones of dogs with prolonged external drainage of the bile. Only the excretion of chlorides in the bile was studied in the present series of cases. The concentration of chlorides in the bile was the same as, or slightly greater than, that in the blood serum, and was seemingly unaffected by such factors as changes in the food, the volume of the bile, or the intake of water or salt by the patient. The total output of chlorides in the bile varied between 1 and 4 grams. This is a quantity of salt that ordinarily would not be significant, although, if no salt were provided in the food, the loss might eventually become significant. The development of cholerrhagia, on the other hand, markedly increased the loss of chlorides; a maximal excretion of 16 grams in one day was observed and one patient lost 87 grams through the biliary tract in the course of a week. This is a quantity that would quickly lead to serious depletion of chlorides in the body if continued unchecked or unless the chlorides were replaced by the administration of appropriate amounts of chlorides, as was done in these cases.

In these cases oral administration of fluids was forced and an additional 1,000 cc. of a solution containing 100 grams of glucose and 10 grams of sodium chloride was given intravenously, so that the daily

intake of fluids varied between 3,000 and 4,000 cc. Notwithstanding this liberal intake of fluids, the output of urine frequently was reduced following the onset of cholerrhagia. This is shown in table 4. Under these conditions, unless care is taken to secure an adequate intake, there is danger of serious depletion of both fluids and salt in consequence of such cholerrhagia.

It is recognized that experiments such as these are open to many criticisms. The collection of bile from a drainage tube, inserted in the gallbladder or common bile duct, is not always complete. When the drainage is through the gallbladder, possible effects of the normal concentrating action of that viscus cannot be excluded. The amount and character of the bile are not the same when there is complete external drainage as when the bile enters the intestine and the normal enterohepatic circulation is uninterrupted.

Most of these criticisms are inherent in the nature of the problem to be studied and cannot wholly be avoided. It is believed, nevertheless, that the changes reported are sufficiently distinct to indicate the response of the liver of man to the relief of biliary obstruction, and some of the modifications of that response caused by various associated conditions.

SUMMARY

The composition of the bile following the relief of biliary obstruction was studied in a series of nine cases.

The total daily output of bilirubin was more or less constant and was not related to the other factors studied in this series of cases. The concentration, on the other hand, varied inversely with the volume of the bile. One or two patients showed some evidence of a washing out of retained pigment, but if this occurred in all it took place so slowly as not to be apparent in observations of as short duration as these.

Biliary obstruction inhibits or stops the formation of bile acids. If the liver is not too greatly injured there is a relatively rapid return to normal; otherwise the return is greatly delayed. This was true both with regard to the concentration and total amount of bile acids.

The concentration of chlorides in the bile is slightly greater than that in the blood serum. With cholerrhagia, the resultant loss of

salts becomes so great as to be of clinical significance. The loss of fluids by this channel may also be so great as to cause diminution in the output of urine.

The concentration of urea in the bile apparently varies directly with that in the blood. Ordinarily this pathway of elimination is not significant, but in one case there was considerable loss of urea through the fistula.

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STUDIES ON THE NITROGEN AND SULPHUR METABOLISM IN "BRIGHT'S DISEASE"

I. THE RETENTION OF NITROGEN AND SULPHUR IN "NEPHROSIS"¹

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For many years the retention of the nonprotein nitrogen in the blood in Bright's disease has been studied in a variety of ways. In recent years the syndrome called "nephrosis" has been described and, by some, considered a clinical entity. For the purposes of the present study, this term has been used to designate patients who showed a syndrome characterized by edema, low basal metabolism, doubly, refractile fat in the urine, the excretion of large amounts of albumin, and the diminution of serum protein with a reversal of the albumin-globulin ratio. None of the patients studied had an increase in the nonprotein nitrogen in the blood. It has been shown that these patients do well on a high protein diet and that they may lose their edema under such conditions. When treated with such a diet they retain large quantities of nitrogen even when given in the form of crystalline urea (1). We have been able to study, over long periods, the metabolic reactions of five patients exhibiting this syndrome. In the present report, we wish to show the relationship between the nitrogen and sulphur retention in these patients.

The subjects of this, and succeeding studies to be reported, were on carefully weighed diets. The food intake was not analyzed, but standard tables of composition were employed to calculate the dietary constituents including the water in the food. To prevent a possible diuretic effect of water the total water intake including the water in the food was kept constant. Refusals of food were weighed on the ward and the total intake calculated by subtraction. The intake was

¹ This work was done under a grant from the Proctor Fund for the Study of Chronic Disease of Harvard University.

essentially constant as to nitrogen, sulphur, phosphorus, calories and water. The diets were varied according to therapeutic necessity in the various patients and corresponded to the standard hospital diets containing 28, 40, 60, 100, and 150 grams of protein. We thus had the advantage of keeping the patient on an approximate diet for a long time before the more laborious process of carefully weighing the foods was commenced. In only one period presented (number 10, fig. 1) was the patient on a weighed diet as little as four days and this patient had been on approximately the indicated dietary level for four weeks. In the remaining periods all patients had been on weighed diets of the

TABLE 1
Additional data on periods shown in figure 1

Period number	Dates of period	Patient number	Total serum protein	Serum albumin	Serum globulin	Blood cholesterol	Basal metabolic rate
			grams per 100 cc.	grams per 100 cc.	grams per 100 cc.	mgm. per 100 cc.	per cent
I	November 12-15, 1928	I	5.0	1.4	3.6		
II	November 19-22, 1928	I	5.1	1.9	3.2	454	-24
III	July 17-20, 1928	V					-11
IV	June 28-July 1, 1928	III	4.6	3.0	1.6	416	-7
V	March 7-10, 1929	IV	3.7	0.4	3.3	471	
VI	March 4-7, 1929	IV	3.4	0.5	2.9	180	-8
VII	July 11-14, 1928	V				290	
VIII	October 7-10, 1928	II	5.5	2.3	3.2	526	-6
IX	June 28-July 1, 1928	V	3.6	1.7	1.9	200	-6
X	May 13-18, 1928	III	5.3	2.5	2.8	312	-10

magnitude presented for at least ten days. All diets contained about 2000 calories made up largely by carbohydrate, as the fat intake never rose above 80 grams. The nitrogen, sulphur and phosphorus ran almost exactly parallel and the levels are sufficiently indicated in figure 1.

Analyses of the urine were done daily for creatinine by the Folin method (2), total nitrogen by the Folin modification of the Kjeldhal method (2) and total sulphur by Fiske's benzidine method (3). The nitrogen of the feces was determined in some experiments, but as this was found to make no significant difference it was not done always. For technical reasons the sulphur of the feces was not determined, but

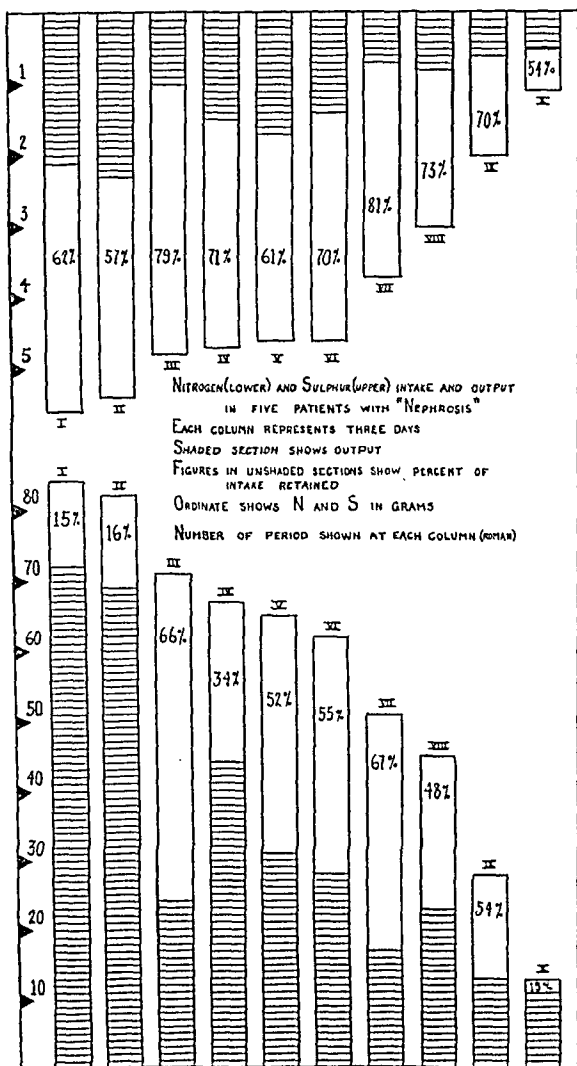


FIG. 1. NITROGEN AND SULPHUR INTAKE AND OUTPUT FOR THE 10 PERIODS SHOWN IN TABLE 1

it has been shown to be insignificant on high protein diets and to run parallel to the nitrogen. In order to minimize day to day variations we have chosen three days as the unit for comparison. All our experiments have been done on this basis. For the present study we have selected ten such periods free from any influences such as changes in diet or others to be reported later. In table 1 the periods presented in figure 1 are correlated with certain chemical findings and from the dates given in this table the general clinical condition of the patient may be ascertained by reference to the brief histories appended. In this connection it is interesting to point out that these periods of study included both edematous and nonedematous subjects.

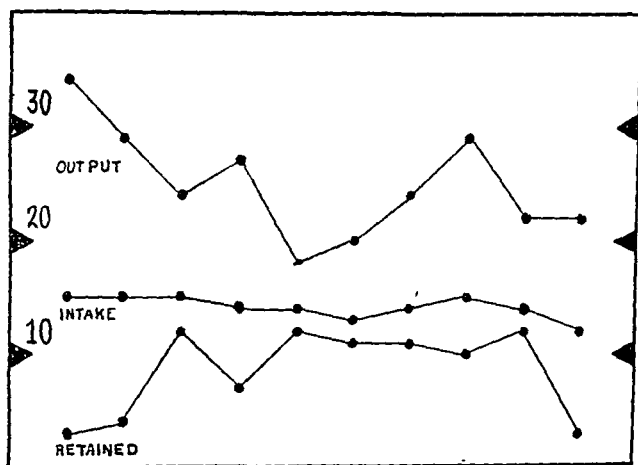


FIG. 2. THE TEN COLUMNS OF RATIOS ARE CALCULATED FROM THE TEN PERIODS SHOWN IN FIGURE 1

The results of this particular study are shown in the accompanying charts. Figure 1 summarizes the intake and output of nitrogen and sulphur of ten three-day periods from five patients on a varying level of protein intake. They are arranged from left to right in a descending series. The columns represent the intake while the shaded portion represents the output. The percentage figures in each column indicate the amount of the intake retained. It is immediately apparent that the percentage of retention of sulphur is greater under any of the circumstances than that of nitrogen. This is contrary to the usual

experience that sulphur is excreted more readily and earlier than nitrogen when protein is added to the diet (4). Blood sulphur determinations were not made, but Wakefield and Keith (5) have shown that in such patients there is no increase in the blood sulphur. The retention of sulphur on the lower dietary levels taken in conjunction with Keith's findings, demonstrate that this retention is not due to a failure of excretion on the part of the kidney.

In this connection, the nitrogen-sulphur ratios are of interest and are shown in figure 2, taken from the results presented in figure 1. It will be noted that the nitrogen-sulphur ratio of the diet corresponds roughly to that in starvation which is about 14 to 1, and this has been assumed to be the ratio in muscle protein (6). It is to be noted, that regardless of the level of the diet, the ratio in the intake remained practically constant. The nitrogen-sulphur ratio in the urine varied considerably, from period to period, but in every case, was significantly higher than that of the intake, and in certain cases yielded very high figures indeed. If we attempt to calculate the nitrogen-sulphur ratio of the "protein" retained, we get the lowest curve on the chart, indicating that such retained protein is rich in sulphur. It will be noted that except at the extremes of dietary intake, the N:S ratio of the retained protein appears to run at a fairly constant level, always below that of the intake. It may be that the variation of the output indicates an attempt to maintain the optimum level in the retained protein.

It is interesting to compare the sulphur-poor nitrogen excretion in these experiments with the descriptions of deposit protein, which is said to be poor in sulphur (4), suggesting that "deposit protein" may be the most important source of the N excreted in these patients. Boothby (7) found comparably high N:S ratios in the urine in patients with myxedema under treatment by thyroxin, and previous work (8) has shown that similar protein may be mobilized in normal individuals by the administration of iodides. It seems undesirable to erect an hypothesis on the basis of the evidence here presented, but these data suggest very strongly that the problem of the nephrosis syndrome may be concerned more with the intermediary sulphur metabolism than with the kidney itself. As to the exact phase of the metabolism involved we have no clue at the present time.

SUMMARY

1. The nitrogen and sulphur excretion of five patients exhibiting the nephrosis syndrome were studied while these patients were on a diet constant as to N, S, P, calories and water.
2. As in other studies a large retention of nitrogen occurred.
3. The retention of sulphur was greater than that of nitrogen regardless of the level of dietary nitrogen.
4. The N:S ratio in the urine was high.
5. The N:S ratio of the "retained protein" was low.

It is a pleasure to acknowledge the assistance of the diet kitchen in these experiments and especially that of Miss Mary Robertson.

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CASE HISTORIES

Case I. O'C. (Medical number 33490), a single, white male of 24 years, first entered the hospital on November 7, 1926 complaining of edema of the legs. His past history was unimportant except that in the preceding 20 months he had had

three periods of edema of the legs, the last occurring in April, 1925. This edema cleared up with dietary treatment alone. The treatment was apparently a low protein diet. At this admission he showed a large soft edema of the legs and a secondary anemia; hemoglobin 50 per cent, red blood cells 4,700,000. The urine showed large amounts of albumin and constantly contained red blood cells. Phthalein excretion was 55 per cent. Blood pressure 135/80. Blood cholesterol was elevated and basal metabolic rate minus 14 per cent. He was treated with desiccated thyroid in large doses without success but improved rapidly on a high protein diet (150 grams). He was readmitted in November, 1927, following a period in which he reduced his diet and again in October, 1928, when edema recurred following a comparatively slight cold. The blood analyses and urine analyses showed no essential change, though the secondary anemia was much improved. Blood pressure remained within normal limits and the phthalein excretion was 45 per cent. Blood urea nitrogen was at all times within normal limits. He was edema free by November 1, 1928.

Case II. Sp. (Medical number 32897), a married white male of 43 years, entered the hospital on July 5, 1928 complaining of swelling of the face and ankles of two weeks duration. On examination there was also found fluid in the abdomen together with enlargement of the spleen and liver. The circulatory system was normal; blood pressure 135/85. Urine showed large amounts of albumin with no pathological elements in the urinary sediment except doubly refractile fat granules. Phthalein excretion was 50 per cent; basal metabolic rate ranged from minus 6 per cent to minus 25 per cent and only on the first examination was the albumin of the blood greater than the globulin. Blood cholesterol was persistently elevated. On high protein diet his edema decreased but did not disappear. Thyroid therapy did not reduce the edema. It was finally found that he remained most free of edema on a diet of 100 grams of protein. In October, 1928, he developed a very slight secondary anemia. He left the hospital in December and re-entered in 1929 with hypertension, and retinal hemorrhages comparatively free of edema. During the period of this study he was only showing puffiness of the eyes in the morning and had slight pitting edema of the shins.

Case III. Z. (Medical number 32464), a single, white female of 23 years, entered the hospital April 21, 1928, complaining of edema of the legs. The onset was insidious beginning with weakness and the edema was first noted 8 months before. Later, edema spread from feet to legs and subocular edema was noted; then nocturia, palpitation and dyspnea on exertion developed. On entry, the patient was pale and there was slight edema of the legs and over the sacrum. Blood pressure 98/70. Wassermann reaction positive. She was treated with a low protein diet and given active antilutetic therapy without amelioration of her symptoms until on June 9th she was given a high protein diet. The urine showed large amounts of albumin with a moderate number of casts in the sediment.

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Phthalein excretion 55 per cent; basal metabolic rate, minus 6 per cent to minus 10 per cent; blood urea nitrogen, 9 mgm. per 100 cc. The serum proteins showed the usual reversed ratio of albumin to globulin. She was practically edema free during period IV and had slight edema during period X.

Case IV. Alg. (Medical number 34316) a married, white female of 26 years, entered the hospital on February 12, 1929, complaining of edema and shortness of breath. The onset was in September, 1928, six weeks after a cold and sore throat. She had been treated at home with digitalis and low protein diet. Examination revealed fluid in both pleural cavities and in the abdomen, edema of the legs and over the sacrum, some pharyngeal inflammation, large amounts of albumin in the urine and reversal of the serum protein ratio. The urinary sediment was not remarkable. Blood pressure 125/85; blood urea nitrogen 11 to 15 mgm. per 100 cc.; blood cholesterol elevated; basal metabolic rate minus 8 per cent. After two days on the Karrell diet she was treated with a high protein diet without much improvement. On April 1st, she developed a sharp pain in the upper abdomen and died on April 4th of a fulminating septicemia.

Case V. R. (Medical number 32820) a married, white female of 38 years, entered the hospital on June 21, 1928, complaining of swelling of the ankles. She had had chorea in childhood and had been known to have rheumatic valvular heart disease since that time. This involved both the mitral and aortic valves. The onset of the present edema was 8 months ago and for the past month she had been in the Boston City Hospital where she was completely digitalized without relief. On entry there were the expected cardiac signs, a fluid wave in the abdomen and pitting edema over the ankles. Vital capacity, 45 per cent; blood pressure, 180/80; phthalein, 10 per cent. The urine contained large amounts of albumin and a moderate number of granular casts. There was severe secondary anemia; red blood cells, 2,500,000; basal metabolic rate minus 6 per cent. Serum proteins showed reversed ratio and blood cholesterol was elevated. She became edema free on August 13th after thorough digitalization and high protein diet. Infected teeth were removed on July 21st. This was followed by oliguria until the condition of the mouth again permitted the mastication of the high protein diet on August 1st. She died months after this period with the signs of renal failure.

THE EFFECT OF ATROPINE UPON GASTRIC SECRETION AFTER HISTAMINE STIMULATION

By W. SCOTT POLLAND

(From the Department of Medicine, Stanford University Medical School, San Francisco)

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It is generally assumed by physicians that atropine decreases gastric secretion, and although many studies have been made on animals and man, few exact details as to its action in man are known. Histamine, although studied extensively by pharmacologists and physiologists, since the work of Dale and Richards (1), has only recently interested the clinician. This has come about as a natural development of the work of Popielski (2, 3), who found that histamine was a powerful stimulant of gastric secretion in dogs, and later of Carnot, Koskowski and Libert (4), who described its action on the stomach in man. Bloomfield and Polland (5) have recently described a method by which it has been found to be of diagnostic value in studies on gastric secretion. It seemed advisable to study the effect of atropine on the gastric secretion caused by histamine in man. It was thought that such studies might enable one to estimate more accurately the value of histamine as a stimulating agent, and at the same time throw some light on the more fundamental problems of gastric secretion.

LITERATURE

Keeton, Luckhardt and Koch (6) using dogs with Pavlov pouches or gastric fistulae showed that there was an antagonism between histamine and atropine. In their experiments, even after temporary inhibition by atropine, histamine always produced a reappearance of secretion. They noted that if atropine was given after histamine, there was first a fall in the quantity of secretion and pepsin, followed later by a reduction in acid. They suggested that this indicated a different secretory mechanism for pepsin and acid. No similar studies have been made on man.

As regards the effect of atropine on gastric secretion, the literature abounds with conflicting and contradictory reports. A review of the bibliography is beyond the scope of this paper. Altshuler (7) has recently surveyed the experimental work, and attempted to reconcile contradictory observations on the basis that the action of drugs depends upon what phase of activity the cells are in when the stimulus reaches them. He believes that the same drug acting on the same cell might at one time result in stimulation, and at another in inhibition.

METHODS

Exactly the same procedure was used as has been previously described (5), except that after the volume of secretion and titratable acidity had practically returned to normal, a second injection of histamine (0.1 mgm. per 10 kilograms body weight) and atropine (0.2 mgm. per 10 kilograms body weight) was administered subcutaneously. The response to histamine alone was taken as a control for the subsequent effect of histamine and atropine. Previous studies in this laboratory (8) have shown that repeated injections of histamine that follow after the effect of a former injection has worn off, produce practically the same titratable acidity and volume response. The dose of atropine used was sufficient to produce mild toxic symptoms—dryness of mouth and blurring of vision.

The following determinations were made on the various ten-minute specimens: (a) volume, (b) titratable acidity, and (c) pepsin. Titratable acidity was determined with di-methyl and phenolphthalein in the usual way, and pepsin according to the method of Polland and Bloomfield (9).

MATERIAL

Seven patients were studied. Four of these presented no evidence of organic disease of the stomach; three were cases of duodenal ulcer. Volume and titratable acidity were measured in all, and pepsin was determined in 4 cases. Table 1 shows a complete protocol to illustrate the procedure.

RESULTS

Case 1. R., a man, age 21 with evidence of mental retardation, had never had any symptoms of gastric disease. The results of the examination are shown in

chart 1. The control period showed a secretion of normal volumes and titratable acidity. At the end of the period both had practically returned to the fasting level. After the administration of histamine and atropine, there was a brisk temporary rise in the volume per ten-minute period followed by a fall below that of the fasting level. On the other hand, the titratable acidity¹ mounted to a level much higher than with histamine alone, and remained relatively high even when the volume of secretion was low. In spite of the rise in titratable acidity, the total output of acid fell with the volumes.

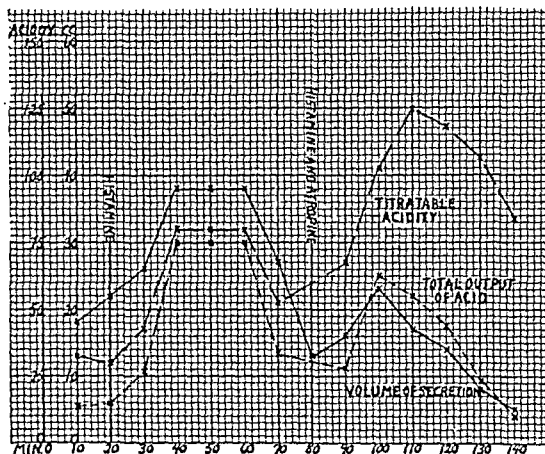


CHART 1. CURVES OF VOLUME OF SECRETION, TITRATABLE ACIDITY, AND TOTAL OUTPUT OF ACID FROM CASE 1 AT TEN-MINUTE INTERVALS AFTER HISTAMINE, AND AFTER HISTAMINE AND ATROPINE

Case 2. G. B., a man, age 42, had symptoms of mild indigestion for ten years. Physical examination was negative. Stools were negative for occult blood. X-rays showed no evidence of disease of the stomach. His symptoms partially subsided after a short stay in the hospital. The control period showed secretion

¹ Throughout this paper, titratable acidity refers to the number of cubic centimeters of $N/10$ NaOH necessary to neutralize 100 cc. of gastric juice with phenolphthalein as an indicator. The total output of acid represents the total amount of acid secreted per ten-minute period expressed as $N/10$ HCl.

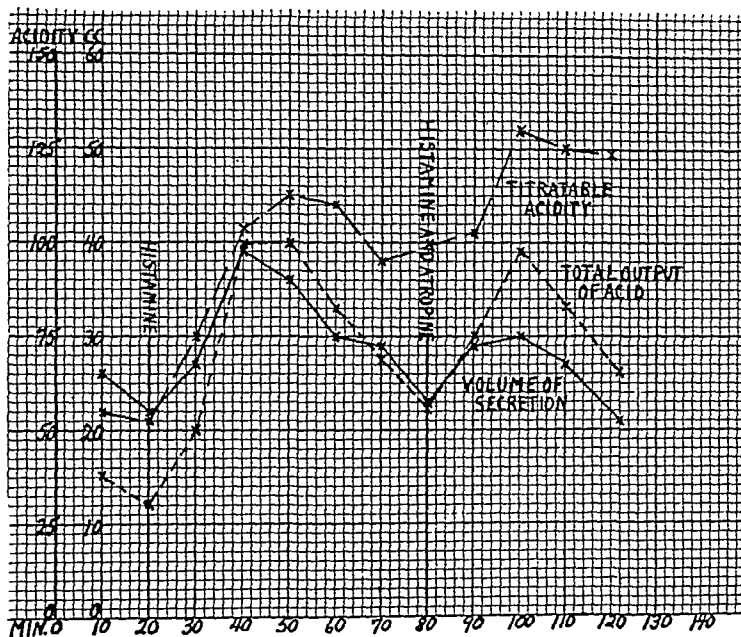


CHART 2. CURVES OF VOLUME OF SECRETION, TITRATABLE ACIDITY, AND TOTAL OUTPUT OF ACID FROM CASE 2 AT TEN-MINUTE INTERVALS AFTER HISTAMINE, AND AFTER HISTAMINE AND ATROPINE

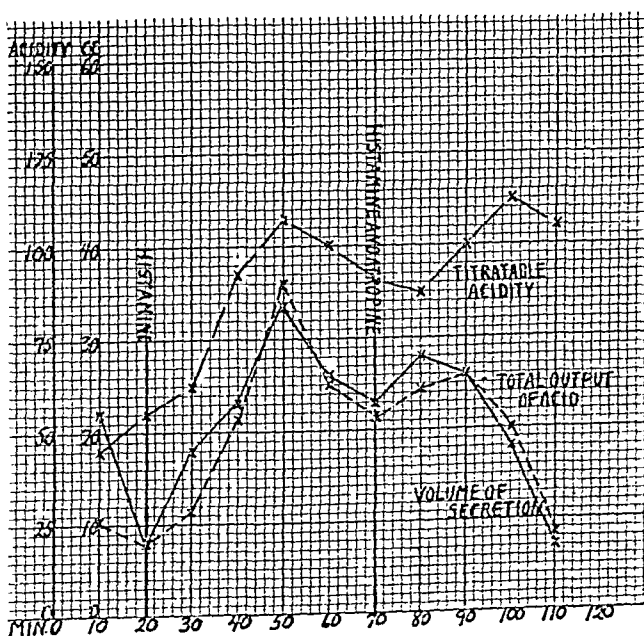


CHART 3. CURVES OF VOLUME OF SECRETION, TITRATABLE ACIDITY, AND TOTAL OUTPUT OF ACID FROM CASE 3 AT TEN-MINUTE INTERVALS AFTER HISTAMINE, AND AFTER HISTAMINE AND ATROPINE

of normal amounts of juice of normal titratable acidity (chart 2). After histamine and atropine, there was a transient rise of the volume per ten-minute period, followed by a fall. Simultaneously, there was a marked rise in the titratable acidity, but the total output of acid was diminished.

Case 3. A. M., a woman, age 48, had symptoms of indigestion relieved by food or soda for seven years. Physical examination and laboratory studies were essentially negative. Gastro-intestinal x-rays showed a constant constriction of

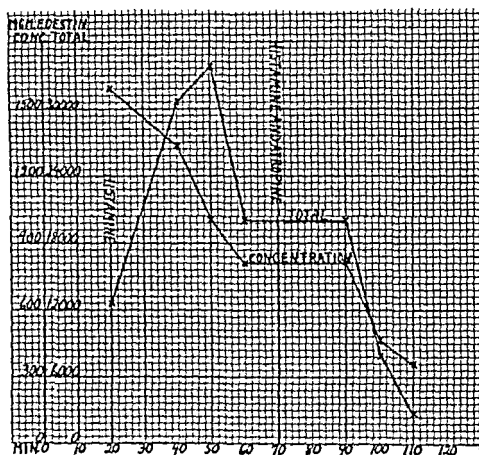


CHART 4. CURVES OF CONCENTRATION AND TOTAL OUTPUT OF PEPSIN FROM CASE 3 AT TEN-MINUTE INTERVALS AFTER HISTAMINE, AND AFTER HISTAMINE AND ATROPINE

the duodenum, and the x-ray diagnosis was duodenal ulcer. The control examination showed the volumes and titratable acidity were within normal limits (chart 3). After histamine and atropine the titratable acidity rose while the volumes fell. Even after the volume had fallen to 7 cc. per ten-minute period, the titratable acidity remained high—105 cc. N/10 HCl per 100 cc. of juice. The total output of acid was, as usual, decreased. Chart 4 shows the effect of histamine and atropine upon pepsin. (Pepsin is expressed in terms of the number of milligrams of edestin digested by one cubic centimeter of gastric juice in 30 minutes.) The control period shows, as has been previously noted (10), a fall in concentration but a

transient rise in total output of pepsin after histamine. After histamine and atropine, there is a marked fall in both the concentration and total output of pepsin for each ten-minute period.

Case 4. J. G., a boy, age 17, entered the hospital for minor complaints and showed no evidence of organic disease. The control volumes and titratable

TABLE 1
Complete protocol of a typical experiment (Case 4)

Number of specimen	Time	Amount	Character	Acid titratable			Pepsin	
				Free	Total	Output per period	Edestin digested by 1 cc. of juice	Total edestin digested per 10 minute period
	<i>p.m.</i>	<i>cc.</i>		<i>cc. N/10 per 100 cc.</i>	<i>cc. N/10 per 100 cc.</i>	<i>cc. N/10</i>	<i>mgm.</i>	<i>mgm.</i>
1	1:50	60	Fasting contents. Water clear, small amounts mucus	22	33	19.8		
2	2:00	23	Water clear, small amount mucus	46	56	12.88		

Histamine 0.6 mgm.

3	2:10	30	Water clear	81	88	26.4	1,910	57,300
4	2:20	39	Water clear	94	102	39.78		
5	2:30	33	Water clear	108	118	38.94	899	29,700
6	2:40	22	Water clear	98	112	24.64		
7	2:50	17	Water clear	62	83	14.11	667	11,340

Histamine 0.6 mgm., atropine 1.2 mgm.

8	3:00	16	Water clear	84	100	16.0	1,150	18,410
9	3:10	20	Water clear	116	128	25.6	684	13,680
10	3:20	17	Water clear	128	137	23.29	522	8,901
11	3:30	14	Water clear	138	146	20.44	424	5,980

acidity were normal (table 1 and chart 5). After histamine and atropine, the volumes and total output of acid fell, while the titratable acidity rose as in cases 1, 2 and 3. Chart 6 shows that after histamine there is a fall in the concentration and total output of pepsin, followed, after histamine and atropine, by a slight rise, then a further fall of both.

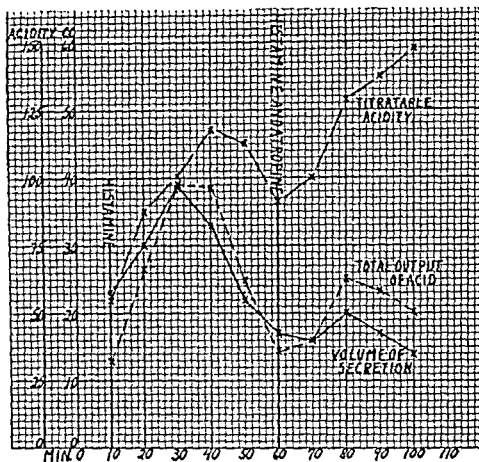


CHART 5. CURVES OF VOLUME OF SECRETION, TITRATABLE ACIDITY, AND TOTAL OUTPUT OF ACID FROM CASE 4 AT TEN-MINUTE INTERVALS AFTER HISTAMINE, AND AFTER HISTAMINE AND ATROPINE

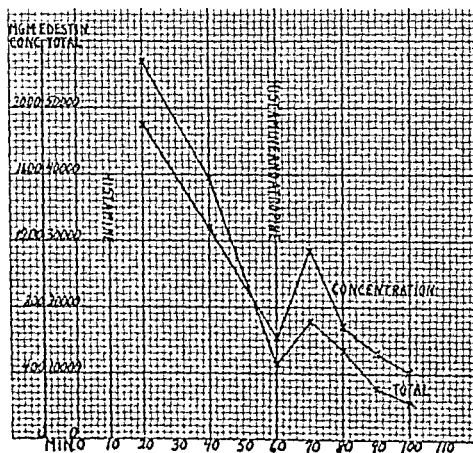


CHART 6. CURVES OF CONCENTRATION, AND TOTAL OUTPUT OF PEPSIN FROM CASE 4 AT TEN-MINUTE INTERVALS AFTER HISTAMINE, AND AFTER HISTAMINE AND ATROPINE

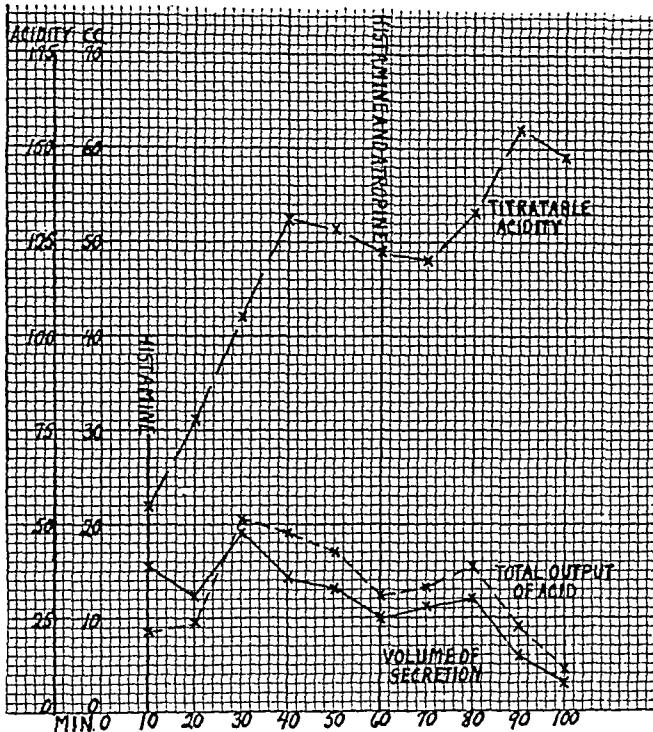


CHART 7. CURVES OF VOLUME OF SECRETION, TITRATABLE ACIDITY, AND TOTAL OUTPUT OF ACID FROM CASE 5 AT TEN-MINUTE INTERVALS AFTER HISTAMINE, AND AFTER HISTAMINE AND ATROPINE

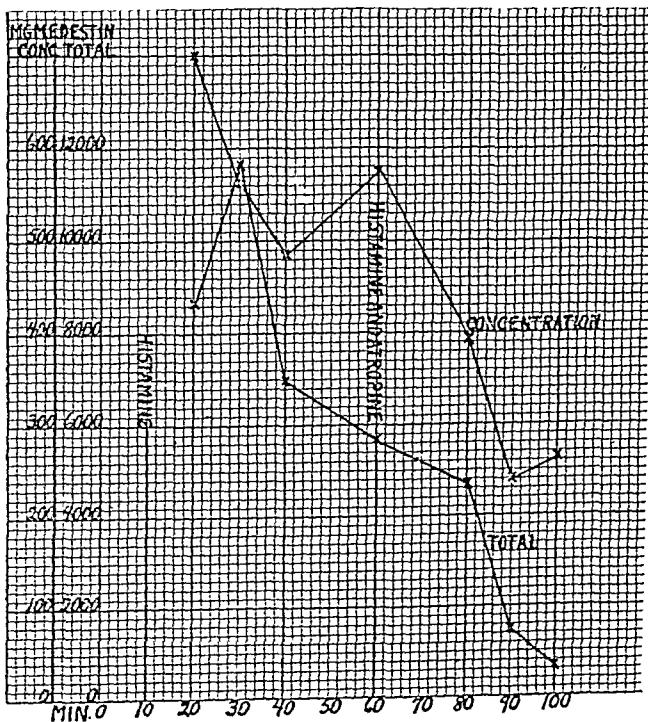


CHART 8. CURVES OF CONCENTRATION, AND TOTAL OUTPUT OF PEPSIN FROM CASE 5 AT TEN-MINUTE INTERVALS AFTER HISTAMINE AND AFTER HISTAMINE AND ATROPINE

Case 5. L. L., a woman, age 30, entered the hospital because of nervousness. No evidences of organic disease of any kind were found. The volume response to histamine was low, but the titratable acidity was normal (chart 7). After histamine and atropine, the volumes fell to the very low level of 3 cc. per ten-minute period, while the titratable acidity reached the remarkable level of 155. The total output of acid fell with the volumes. Both the concentration and total output of pepsin fell markedly after histamine and atropine (chart 8).

TABLE 2
Data from case 6

Number of specimen	Time	Amount	Character	Titratable acidity		Pepsin	
				Free	Total	Edestin digested by 1 cc. of juice	Total edestin digested per 10 minute period
	p.m.	cc.		cc. N/10 per 100 cc.	cc. N/10 per 100 cc.	mgm.	mgm.
1	2:25	30	Fasting contents. Thin, colorless, some mucus	40	58		
2	2:35	38.5	Thin, clear	84	94	1,485	57,190
3	2:45	36	Thin, clear	94	102		
4	2:55	28	Thin, clear	102	112	1,770	49,500
5	3:05	25	Thin, faint brownish tinge (blood)	106	116		
6	3:15	31	Thin, faint brownish tinge (blood)	104	114	1,615	50,400
7	3:25	36	Thin, faint brownish tinge (blood)	80	90		
Histamine 0.7 mgm., atropine 1.4 mgm.							
8	3:35	31.5	Thin, faint brownish tinge (blood)	112	122	1,770	54,900
9	3:45	33	Thin, faint brownish tinge (blood)	124	132		
10	3:55	37	Thin, faint brownish tinge (blood)	126	134	788	27,160
11	4:05	33.5	Thin, faint brownish tinge (blood)	124	134		

Case 6. J. M., a man, age 54, entered the hospital for treatment of a duodenal ulcer of many years duration. His fasting secretion was very high—194.5 cc. of juice per hour, having an average titratable acidity of 105 (table 2 and chart 9). After histamine and atropine, the rate of secretion remained about the same, but the titratable acidity rose from 116 to 134. The concentration of pepsin fell from 1770 milligrams to 788 milligrams edestin digested by 1 cc. of juice (chart 10), and the total output of pepsin fell from 57,190 mgm. to 29,160 mgm. edestin digested per ten-minute period.

ATROPINE AFTER HISTAMINE STIMULATION

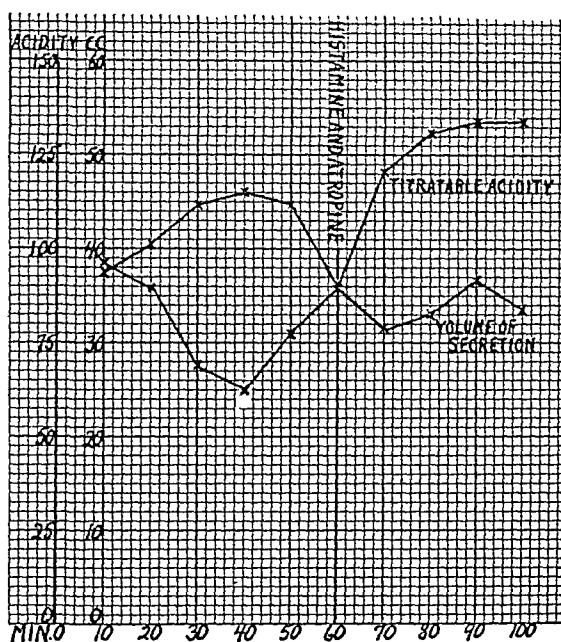


CHART 9. CURVES OF VOLUME OF SECRETION, AND TITRATABLE ACIDITY FROM CASE 6 BEFORE AND AFTER HISTAMINE AND ATROPINE

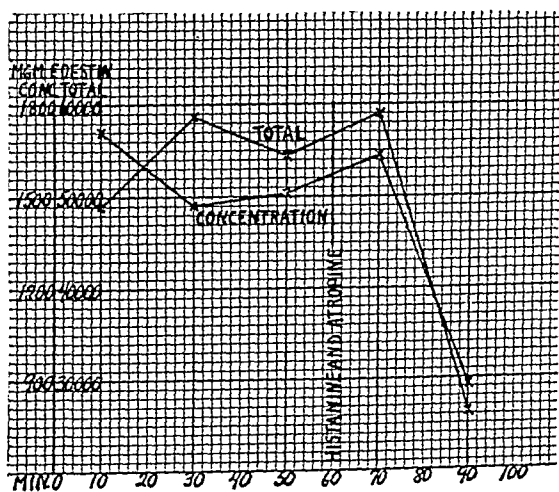


CHART 10. CURVES OF CONCENTRATION AND TOTAL OUTPUT OF PEPSIN FROM CASE 6 AT TEN-MINUTE INTERVALS BEFORE AND AFTER HISTAMINE AND ATROPINE

Case 7. F. A., a man, age 30, had symptoms of indigestion for 20 months. The x-ray diagnosis was duodenal ulcer and his symptoms promptly subsided on a modified Sippy regime. After histamine, the volumes and titratable acidity were high (chart 11). After histamine and atropine, the usual response occurred; the titratable acidity rose while the volumes fell. After the volume had reached a

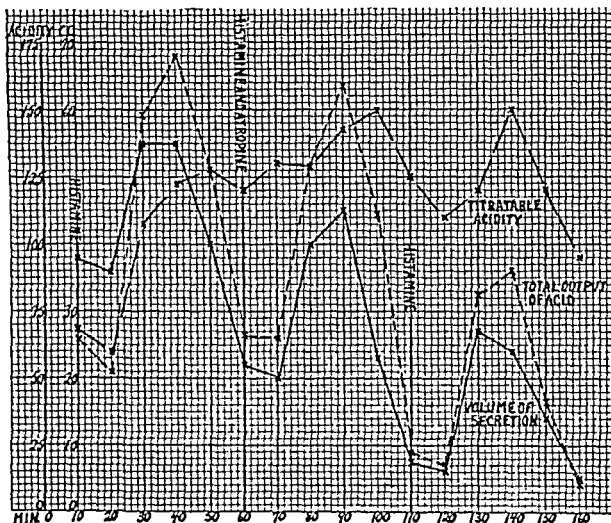


CHART 11. CURVES OF VOLUME OF SECRETION, TITRATABLE ACIDITY, AND TOTAL OUTPUT OF ACID FROM CASE 7, AT TEN-MINUTE INTERVALS AFTER HISTAMINE, AFTER HISTAMINE AND ATROPINE AND AFTER A SECOND INJECTION OF HISTAMINE

secretory rate of 7 cc. per ten-minute period, another dose of histamine was administered. Although the volumes reached 27 cc. per ten-minute period, this was less than half the first response to histamine. However, the titratable acidity rose to a maximum of 150, as compared to the previous response to histamine of 128.

DISCUSSION

A survey of the foregoing charts shows several striking facts:

1. *Volume of secretion.* It is immediately apparent that the volume of secretion is smaller after histamine and atropine, than with histamine alone. In the control period, the output reached its maximum in 20 to 30 minutes and then began to fall, reaching the pre-histamine level in about 50 to 60 minutes. On the other hand, after histamine and atropine, there was usually a slight rise in the volume, followed by a marked fall. Even a second dose of histamine (case 7) failed to produce the normal volume response, while the effect of atropine was still present. These facts are shown quantitatively in table 3.

TABLE 3

Comparison of volume of secretion, average titratable acidity, and total acid output in the first 40 minutes after histamine, and after histamine and atropine

Case	Volume secreted		Titratable acidity		Total output of acid	
	Histamine	Histamine and atropine	Histamine	Histamine and atropine	Histamine	Histamine and atropine
	cc.	cc.	cc. N/10 per 100 cc.	cc. N/10 per 100 cc.	cc. N/10	cc. N/10
1	140	71.5	61	93	88.5	67.08
2	132	107	100	120.5	133.47	128.61
3	101	79	90.5	101.3	95.02	78.15
4	124	67	105	127.8	129.76	85.33
5	59.5	33	111.3	139	66.93	44.17
7	186	128	105	137.5	200.4	175.6

2. *Acidity.* Keeton, Luckhardt and Koch pointed out that in dogs the volume of secretion after atropine diminished more rapidly than the titratable acidity. The present experiments show that in man the titratable acidity rises after histamine and atropine, often to extremely high levels. This is in striking contrast to the volume of secretion. In case 5, for example, the titratable acidity reached 155, while the volume fell to 3 cc., and in case 3 the titratable acidity was 105, while the volume was 7 cc. This complete dissociation of the usual coordinated response to histamine suggests that water and acid are secreted by different mechanisms. If the curves for titratable acidity and volume of secretion are alone considered, it would appear

that the increase in concentration of acid after histamine and atropine was due only to a decrease in volume of water secretion, and that there was no direct effect upon acid secretion. However, if the total output of acid is studied, it is clearly seen that atropine has also a definite inhibitory action on acid secretion. In table 3 is a summary of the actual quantitative relations.

It is believed that the present observations explain why most authorities have found a diminution in the titratable acidity after the administration of atropine. If bread, water, gruel, beef broth or alcohol are used to study gastric secretion, no true index of titratable acidity can be obtained because of the diluting effect of the meal itself. If the actual concentration of acid is higher, but the total output is lower for any given period of observation, the introduction of a diluting agent will give a false idea of the true concentration of the acid. Obviously, this error does not occur if pure gastric juice is studied, such as is obtained after histamine stimulation. However, it is also important to remember that the study of the concentration alone of a given substance is insufficient, and often misleading. Unless one considers the total output, or rate of secretion of a given substance, no true index of the functional state of the stomach can be obtained. This is strikingly shown in all the cases, if the titratable acidity was alone considered, and the total output disregarded. In that case the conclusion would have to be that atropine always augmented acid secretion after histamine. Certainly this conclusion is false when one considers the total output of acid.

3. *Pepsin*. A survey of the pepsin curves shows that after histamine and atropine, the concentration is decreased. The effect on pepsin is more strikingly shown if the total output is considered. The latter seems to parallel the fall in volume of secretion. However, in case 6 the concentration and total output of pepsin fell before the volume of secretion was affected; in cases 4 and 5 the concentration and total output were falling while the volume of secretion was rising. Previous studies (11) have suggested that pepsin and water are not secreted by the same mechanism, and the present study is additional evidence in favor of such a theory. It is clear that the secretion of pepsin has no relation to the secretion of acid.

SUMMARY

Curves are presented comparing the course of gastric secretion after histamine with that after histamine and atropine. The volume of secretion, titratable acidity, concentration of pepsin, total output of acid and total output of pepsin have been studied. Atropine dissociates the normal response to histamine, producing a marked decrease in the volume of secretion, and in the concentration and total output of pepsin. The titratable acidity rises as a result of the only slight inhibitory effect of atropine on the total output of acid.

The results suggest that water, acid and pepsin may have different mechanisms of secretion.

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BLOOD VOLUME AND PLASMA ELECTROLYTE CHANGES IN THE DEHYDRATION OF INFANTS

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In a general review of the subject of anhydremia published in 1923, Marriott (1) brought out several of the phenomena which had been shown to accompany severe clinical dehydration: an increase in the proportion of the total solids and in the dried weight of a unit volume of blood, an increase in its specific gravity, in red cell count and hemoglobin, and in the concentration of the serum proteins. He showed also, however, that when deprivation of fluid, either from lack of intake, from vomiting, or from diarrhea, persisted over a long period of time, a lowering of total solids, red count, and hemoglobin ensued, which was attributed to breakdown of body protein with the liberation of bound water. All of these phenomena might occur at a time when the plasma volume was subnormal, and consequently an investigation of the concentration of these substances in a unit volume of blood was shown to be of limited value in the study of the water economy as a whole in dehydration. Although these considerations emphasized the fatuity of placing reliance on the indirect evidence afforded by changes in the concentration of blood constituents and the necessity for direct investigation of blood and plasma volume changes in dehydration, such direct determinations have been singularly infrequent. Marriott mentioned some in a general way, but did not fully report his data.

A number of studies made since 1923—for example, those of Hartmann (2) and of Hamilton, Kajdi and Meeker (3)—on the serum or plasma concentration of electrolytes in dehydration accompanying the infections and digestive disorders of infants have shown that a lowering of the total electrolyte concentration is, in fact, rather more com-

mon than an increase; and even when dehydration was brought about experimentally by reduction of the water intake, Schiff, Bayer, and Fukuyama (4) were unable to change the concentration of serum total fixed base beyond the limit of error of the method used. If, then, a lowering of serum electrolyte concentration of this kind, or even if no change in concentration occurs, one must abandon the concept that loss of water alone from the blood is the primary event in the development of dehydration, since this would only act to bring about a higher concentration of electrolytes. This actual lowering of serum electrolyte concentration in dehydration appeared to Hartmann (2) of sufficient importance to cause him to postulate that loss of electrolyte from the circulation might occur to an extent that required the body to retain nonprotein nitrogen constituents for their osmotic action so that the normal osmotic pressure of the plasma might be maintained. If water alone had been lost to excess, no such difficulty would have existed, for the osmotic pressure of the plasma would by that measure be increased. The lowering of serum electrolyte concentration could theoretically be brought about by dilution of the blood, though in view of the loss of body weight and the obvious loss of water from the body as a whole, this would seem unlikely; or it could result from loss of electrolyte from the circulation. The latter naturally appeared to be the more plausible explanation; but the true answer to the question is not to be obtained by determinations of concentration alone.

Darrow and Buckman (5) attacked this problem by making blood and plasma volume determinations, together with measurements of serum chloride, bicarbonate, protein, conductivity, and freezing point, in patients with dehydration. Their most valuable data were obtained in a small number of patients who, after the initial determination during severe clinical dehydration, responded well to therapy and were subjected to a second analysis from four days to about five weeks later. These patients all showed during the period of dehydration, as compared with values obtained after recovery, a diminution of whole blood, plasma and cell volumes, and a diminution in total circulating serum chloride and protein; while from the point of view of concentration of dissolved substances in the serum during dehydration there was no constant change in chloride, an increase in bicarbonate, and a somewhat less marked increase in protein, with no essential

change in serum conductivity or freezing point. In the process of recovery they showed that an increase in the total plasma volume was accompanied by, and presumably brought about by, an increase in total plasma water, chloride, and protein in the proportions in which these substances are present in normal plasma. They therefore suggested that the expression "oligemia" more accurately depicts the blood changes accompanying the clinical picture of dehydration. As an explanation of the mechanism of the blood volume and electrolyte changes accompanying the dehydration associated with diarrhea in infants, however, their material as just quoted must be interpreted with some reserve, since two of their patients were older children with encephalitis, one an infant of 16 months with celiac disease, and only one belonged strictly to the clinical class of infantile diarrhea.

We have been able to add to the information pertinent to this subject by further studies of blood and plasma volume, with simultaneous determinations of serum electrolyte concentration, during clinical dehydration in infants with diarrhea and after recovery.

METHODS

Infants with dehydration, manifested by loss of skin turgor, dryness of mucous membranes, depression of the fontanel, and recession of the eyeballs, were subjected immediately on admission, and before any parenteral fluid had been given, to the withdrawal of about 10 cc. of blood for estimation of serum electrolyte concentration, and to determination of the blood volume by the carbon monoxide method (6). Hematocrit determinations, on which the calculation of plasma volume was based, were made in a few instances by the Van Allen method (7) but in the majority of cases by the heparin method of Went and Drinker (8). Serum total base concentration was determined by Fiske's method (9), chloride by the method of Fiske and Sokhey (10), bicarbonate in 0.2 cc. samples by Van Slyke and Neill's method (11), and protein by the method of Greenberg (12). These data permitted the calculation of plasma and cell volumes, and of the total amount of circulating fixed base, chloride, bicarbonate, protein anions, and by difference the undetermined acid fraction, comprising mainly the organic acids, phosphates, and sulphates. In the calculation of bicarbonate, it was assumed that all of the carbon dioxide

liberated in the customary determination of the carbon dioxide combining power was present as bicarbonate ions, regardless of serum pH; the inaccuracy so introduced involves no significant error in the final calculations. For the calculation of the proportion of the total protein present as anions, the formula of Van Slyke, Hastings, Hiller, and Sendroy (13) was used, assuming an albumin globulin ratio of 1.6 and a pH of 7.34; here again, no significant error is introduced by basing our calculations on these assumptions. It was further assumed that determinations of *serum* electrolyte concentration could be applied to calculations of *plasma* electrolyte content without altering their significance. Following these determinations, the patients were treated symptomatically for the relief of dehydration through attention to the underlying infection and by the parenteral administration of normal salt solution, occasionally also with the use of glucose solutions or blood transfusions. At the end of a week or more, when clinical signs of dehydration had completely disappeared, the analytical procedures were repeated in order to obtain figures that might be regarded as normal standards for these individuals. The diet during the period of treatment was not uniform, but in the great majority of cases consisted of a mixture of whole milk with 8.5 per cent of cane sugar added, given first, after a preliminary starvation period of approximately 24 hours, in amounts yielding 20 calories per kilogram per day and increased by daily steps of 10 to 20 calories per kilogram per day up to full caloric requirements. All patients were given as much water by mouth as they could be induced to take; occasionally normal salt solution or 3 per cent glucose solution was substituted for tap water.

The error of the carbon monoxide method of blood volume determination cannot be checked in any entirely satisfactory way, but from theoretical considerations it is estimated to be less than 8 per cent, and the same figure probably applies also to plasma volumes determined by this method. The chief source of inaccuracy is undoubtedly the fitting of the breathing mask to the patient's face and the problem of keeping it adjusted. This being so, we have been satisfied to forego attempts to increase the accuracy of other phases of the determination at the expense of the removal of larger amounts of the patient's blood. On these grounds we have assumed an average value, based

on 54 determinations in normal individuals, for the CO content of the infant's blood prior to the administration of CO in the course of the test, and we have only single determinations of the blood CO concentration at the end of the test. The effect of the chief errors of the procedure is to give false high blood volume figures, and we must admit the possibility that some of our results err in this direction; on the other hand, a false low result is most unlikely.

CLINICAL PROTOCOLS

There were 11 patients in the series, ranging in age from 2 to 14 months at the time of admission.¹ All of them had diarrhea, most of them showed some form of parenteral infection, while a few had bacillary dysentery. Most of them showed some degree of vomiting. The severity of dehydration was graded from 1 to 4, corresponding roughly to a scale of "slight," "mild," "moderate," and "severe." A few of the patients gave perhaps mild but typical pictures of "acute intestinal intoxication;" this applies to cases 1, 2, 4, 5, 9 and 11. The order of arrangement of cases in the following series is based on the change found in plasma volume during dehydration.

Case 1. L. G., girl, age 8 months, had had diarrhea with vomiting for a month. She had been nursed for 2 months, then put on a feeding of milk, water, and cane sugar. Admission temperature 38.6°C.; dehydration grade 4; very toxic, almost moribund. It is likely that pneumonia, the signs of which became definite a day or two later, was present at this time. After the first blood analyses, the patient was vigorously treated with fluids by intravenous and intraperitoneal injections of salt solution and by transfusion of 150 cc. of citrated blood. During the course of her illness she developed bilateral otitis media and an acute stomatitis. Parenteral fluids were no longer required after the first week. The second set of blood determinations was made 11 days after admission. (Fig. 1.)

Case 2. J. K., boy, age 2½ months, had had vomiting and diarrhea for a little over 24 hours, with convulsions occurring shortly before admission. He had been fed since birth on a weak dilution of condensed milk, lately one teaspoon in four ounces of water, and had been offered the breast once a day. Admission temperature 39.5°C.; dehydration grade 4; toxic behavior; no demonstrable infection. After determination of blood volume and serum electrolyte concentration, he was given normal salt solution intraperitoneally and intravenously, and from time to time during the first eight days intraperitoneal and subcutaneous injections were

¹ We have omitted from this report the records of three patients whose cell volume figures were so high as to justify the suspicion of gross leakage of gas in the measurement of blood volume

repeated. The second set of determinations was made 13 days after the first. (Fig. 2.)

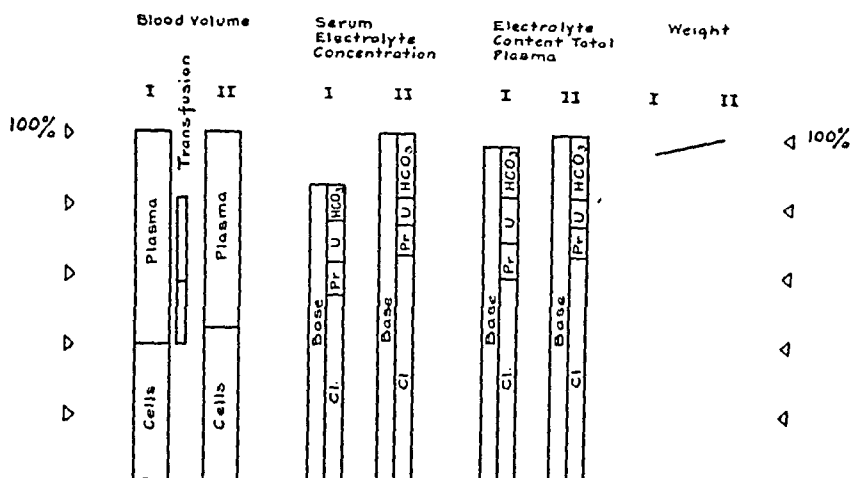


FIG. 1. L. G. AGE, 8 MONTHS. DEHYDRATION, GRADE 4. INTERVAL BETWEEN I AND II, 11 DAYS. TRANSFUSION IMMEDIATELY AFTER I. SALT SOLUTION PARENTERALLY

In all the figures the determinations are represented in an identical way. The first pair of columns depicts total blood volume, separated into cell and plasma volumes. The second pair shows the serum concentration of electrolytes, the height of the columns or subdivisions expressing molar equivalents as cubic centimeters of N/10 solution. Pr = base bound by protein; U = residual anion. The third pair represents the total plasma content of electrolytes in circulation at the time of the test, and is obtained by multiplying the plasma volume by the serum electrolyte concentration. In each of the three pairs of columns, the one on the right, representing determination number II made after recovery, is plotted on a scale chosen to show a 100 per cent value for total blood volume, fixed base concentration in the serum, and total plasma content of fixed base. The results of determination number I, representing the conditions during dehydration, are then plotted to the same scale so that the eye can readily measure the approximate percentage deviation from the recovery value. The curve of weight change is similarly treated, the recovery figure being given the 100 per cent value. Where transfusions have been given between the two determinations, they are plotted in to scale between the blood volume columns. The exact figures for the calculated percentage deviations are reported in tables 1 and 2.

Case 3. M. A., girl, age 5 months, had had diarrhea for 9 days. She had been nursed only four days, and had then been put on whole cow's milk with added

sugar. Admission temperature 36.6°C.; dehydration grade 3; slightly toxic; no demonstrable infection. After the first blood determinations, she was given an

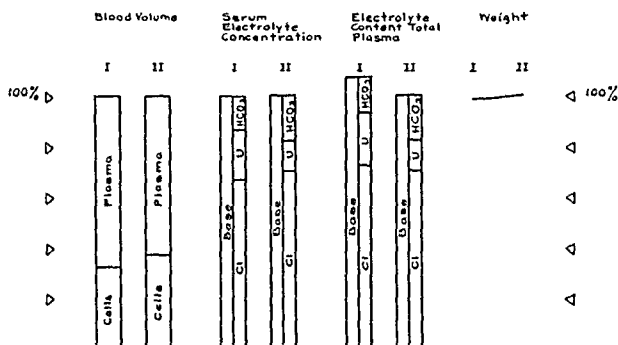


FIG. 2. J. K. AGE, 2½ MONTHS. DEHYDRATION, GRADE 4. INTERVAL BETWEEN I AND II, 13 DAYS. SALT SOLUTION PARENTERALLY. TEMPERATURE 39.5° TO 36.8°C.

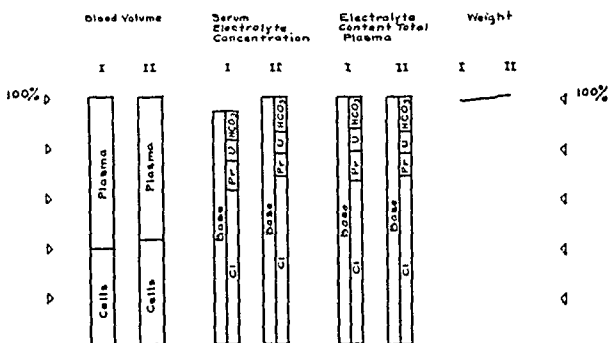


FIG. 3. M. A. AGE, 5 MONTHS. DEHYDRATION, GRADE 3. INTERVAL BETWEEN I AND II, 10 DAYS. SALT SOLUTION PARENTERALLY

intraperitoneal injection of salt solution, which was repeated three days later. The second set of determinations was made 10 days after admission. (Fig. 3.)

Case 4. J. R., boy, age 3 months, had been having diarrhea for three weeks before admission. He had been nursed up to a week before admission, but the breast milk supply was suspected of being inadequate. When symptoms commenced, he was at first given supplemental feedings of protein milk; in the week preceding admission, he had been weaned to bottle feedings of protein milk, buttermilk, and finally barley water alone, without relief of symptoms. Admission temperature 37.0°C .; dehydration grade 4; toxic behavior; congestion of pharynx; thrush; weight 2800 grams, showing that this was an acute illness grafted on a severe chronic nutritional disturbance. After the first set of blood determinations he was given 250 cc. of normal salt solution intraperitoneally and a transfusion of 50 cc. of citrated blood. Parenteral fluid injections were repeated from

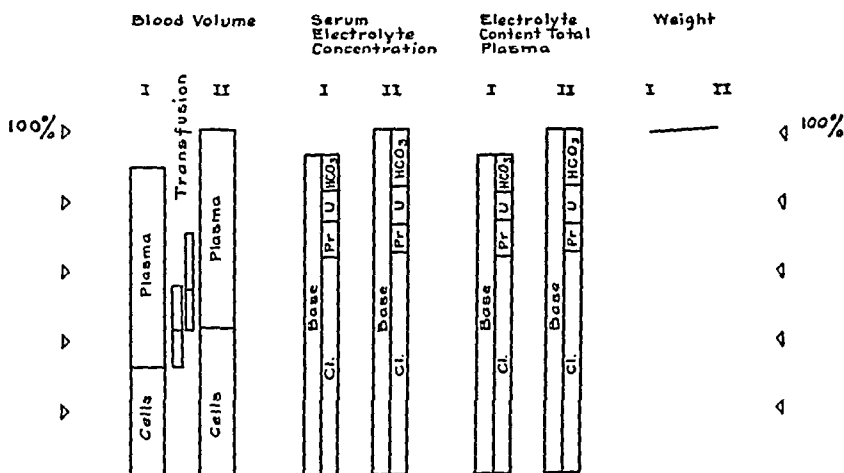


FIG. 4. J. R. AGE, 3 MONTHS. DEHYDRATION, GRADE 4. INTERVAL BETWEEN I AND II, 10 DAYS. TRANSFUSION, 18 CC./KGM. SALT SOLUTION PARENTERALLY

time to time during the first four days, and a second transfusion of 60 cc. was given three days after admission. The second determinations were made after an interval of 10 days. (Fig. 4.)

Case 5. C. F., girl, age $13\frac{1}{2}$ months, had been having diarrhea for 5 days, with fever up to 39.7°C . She had been nursed ever since birth, and had also had a variety of table food since the age of 9 months. Admission temperature 39.2°C .; dehydration grade 2; markedly toxic behavior; bronchopneumonia; B. dysenteriae, Flexner type, isolated from stools. After the first blood analyses, she was given an intravenous injection of 5 per cent glucose solution and a subcutaneous one of normal salt solution. The latter was repeated five times in the next ten days. At the end of two weeks the frequency of the movements had greatly diminished,

all clinical signs of dehydration had disappeared and she appeared to be thriving, although she was still passing mucus and occasionally blood. The second set

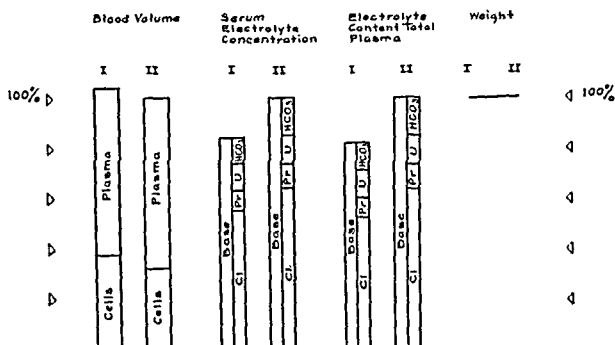


FIG. 5. C. F. AGE 13½ MONTHS. DEHYDRATION, GRADE 2. INTERVAL BETWEEN I AND II, 15 DAYS. GLUCOSE AND SALT SOLUTION PARENTERALLY

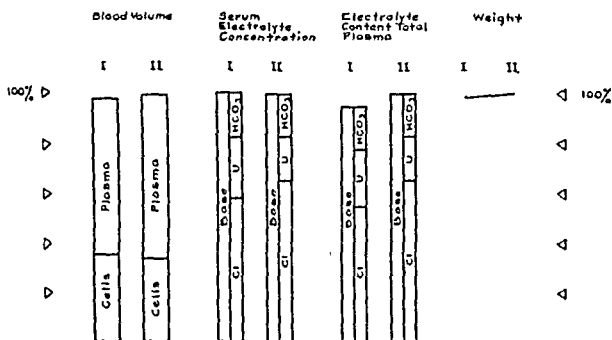


FIG. 6. R. M. AGE 4 MONTHS. DEHYDRATION, GRADE 4. INTERVAL BETWEEN I AND II, 9 DAYS. SALT SOLUTION PARENTERALLY

of determinations of blood volume and electrolyte concentration was made 15 days after admission. (Fig. 5.)

Case 6. R. M., boy, age 4 months, had had diarrhea for 8 days and vomiting for 3 days before admission. He had been nursed for 6 weeks, then fed on condensed milk mixtures. Admission temperature 39.5°C.; dehydration grade 4; moderately toxic behavior; slight degree of congestion of pharyngeal mucous membrane; *B. dysenteriae*, Y type, isolated from stools. After the first blood determinations he was given an intraperitoneal injection of normal salt solution. Parenteral administration of salt solution was repeated three times in the first five days. By the ninth day, when the blood analyses were repeated, the signs of dehydration had disappeared and the diarrhea had greatly diminished in severity. (Fig. 6.)

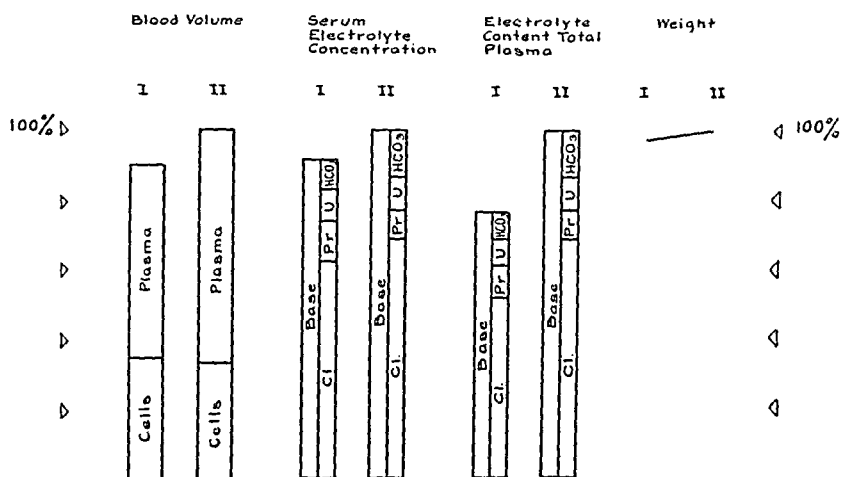


FIG. 7. F. B. AGE, 2 MONTHS. DEHYDRATION, GRADE 3. INTERVAL BETWEEN I AND II, 9 DAYS. SALT SOLUTION PARENTERALLY

Case 7. F. B., girl, age 2 months, had been having diarrhea for two weeks, vomiting for one week. She had been nursed 4 weeks, then put on a milk-water-cane sugar mixture. Admission temperature 37.2°C.; dehydration grade 3; no toxic signs; the appearance of the stools, and the absence of evidence of infection elsewhere, led to a clinical diagnosis of bacillary dysentery which, however, was never substantiated bacteriologically. After the first blood determinations she was given an intraperitoneal injection of normal salt solution, with sufficiently satisfactory effect that it did not require repetition. The second blood determinations were made 9 days after the first. (Fig. 7.)

Case 8. B. J., girl, age 2 months, had had diarrhea for 6 days, vomiting and fever for 4 days. She had been nursed for one month, then put on a mixture of milk, water and cane sugar; she had also been getting orange juice, prune juice,

and a preparation of irradiated ergosterol daily for one month. Admission temperature 38.0°C.; dehydration grade 2; not particularly toxic; respirations slightly increased in depth; no parenteral infection found. After determination of blood volume and serum electrolyte concentration, she was given an intraperitoneal injection of normal salt solution with temporary relief of dehydration. Parenteral administration of salt solution was resorted to five times in the first seven days. No other type of fluid was used, nor was alkali given by mouth. The second set of blood determinations was made 16 days after the first. (Fig. 8.)

Case 9. W. E., boy, age 7 months, had had fever and diarrhea for four days, vomiting for three days. He had been nursed for two months, then put on a

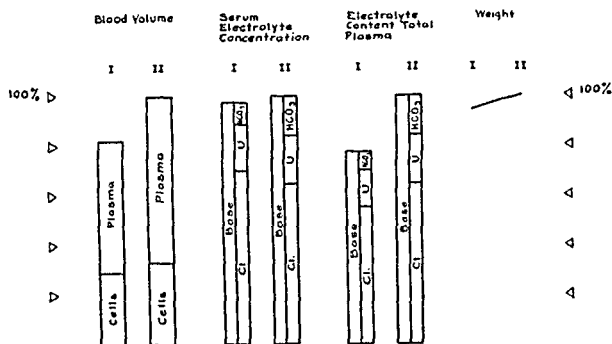


FIG. 8. B. J. AGE, 2 MONTHS. DEHYDRATION, GRADE 2. INTERVAL BETWEEN I AND II, 16 DAYS. SALT SOLUTION PARENTERALLY

mixture of milk, water, and dextrimaltose; recently he had been getting a little supplemental solid food. Admission temperature 38.0°C.; dehydration grade 2; markedly toxic behavior; no parenteral infection found. After the initial determination of blood volume and serum electrolyte concentration, he was given 5 per cent glucose solution intravenously and normal salt solution intraperitoneally. The response was unusually well sustained, no further parenteral fluid administration being required. The diarrhea had subsided by the end of four days. The second set of blood analyses was made 8 days after admission. (Fig. 9.)

Case 10. M. K., girl, age 8½ months, had had diarrhea and marked prostration for three days. She had been nursed for two months; the subsequent feeding history could not be satisfactorily obtained. Admission temperature 37.4°C.;

dehydration grade 2; not markedly toxic at first, but both dehydration and prostration increased in the first five days; pyuria was first discovered four days after

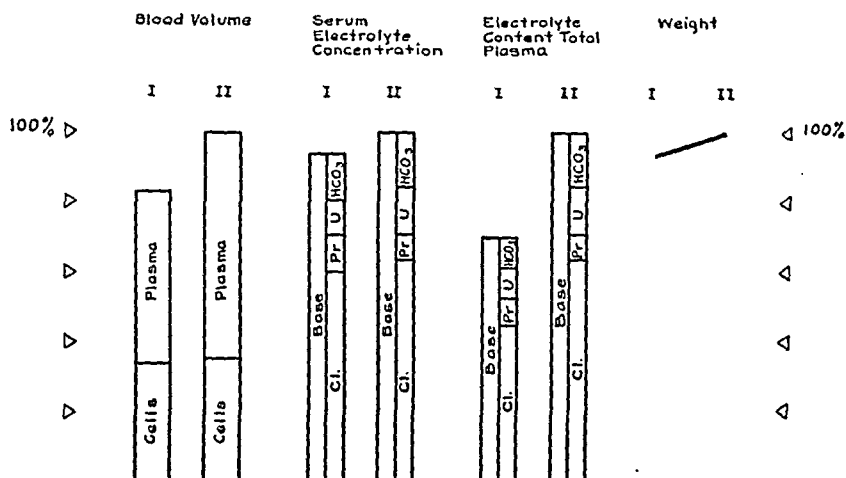


FIG. 9. W. E. AGE, 7 MONTHS. DEHYDRATION, GRADE 2. INTERVAL BETWEEN I AND II, 8 DAYS. GLUCOSE AND SALT SOLUTION PARENTERALLY

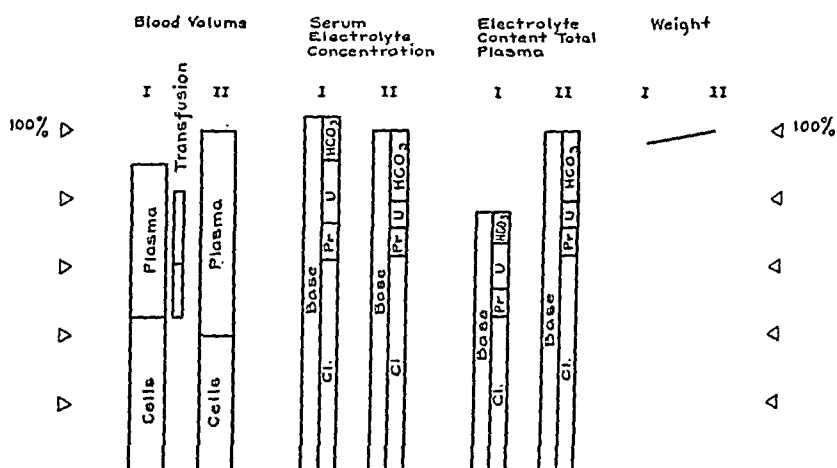


FIG. 10. M. K. AGE, 8½ MONTHS. DEHYDRATION, GRADE 2. INTERVAL BETWEEN I AND II, 17 DAYS. TRANSFUSION 4 DAYS AFTER I. SALT SOLUTION PARENTERALLY

admission. After the first set of blood determinations she was given an intravenous injection of normal salt solution. During the first nine days in the hos-

pital she required parenteral administration of fluids eight times, and a transfusion of 200 cc. of citrated blood was given on the fourth day after admission. Both the diarrhea and dehydration had cleared up at the end of two weeks, and the second set of blood analyses was made 17 days after the first. (Fig. 10.)

The subsequent course of this patient has established the basic diagnosis as pyelonephritis secondary to ureteral stricture. None of the subsequent febrile attacks, of which there have been several, has been associated with dehydration of any severe degree.

Case II. G. V., boy, age 14 months, had had diarrhea for 5 days and vomiting for one day. He had been nursed since birth and had had indefinite amounts of

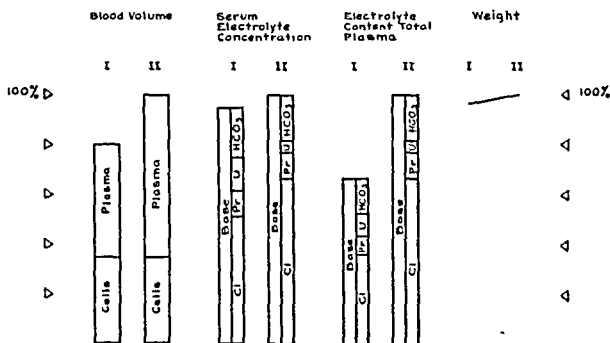


FIG. 11. G. AGE, 14 MONTHS. DEHYDRATION, GRADE 1. INTERVAL BETWEEN I AND II, 23 DAYS. GLUCOSE AND SALT SOLUTION PARENTERALLY

table food in addition. Admission temperature 38.2°C.; dehydration grade 1; in contrast, the toxic behavior was marked; B. dysenteriae, Y type, isolated from the stools. After the first blood analyses, he was given 5 per cent glucose solution intravenously and normal salt solution intraperitoneally. Although the toxic symptoms cleared up quickly, diarrhea and dehydration persisted for several days. A week after admission the skin and subcutaneous tissues showed a more marked degree of dehydration than on admission; yet the patient spent a good part of the day standing up in his crib. Parenteral administration of salt solution was repeated on the ninth day. The second set of blood analyses was made 23 days after the first. (Fig. 11.)

TABLE 1
Blood, plasma, and cell volume changes, body temperature and weight

Case number	Interval between I and II		Circulating blood volume			Blood volume per kgm.			Total circulating plasma volume			Plasma volume per kgm.			Total circulating cell volume			Cell volume per kgm.			Body temperature			Weight		
	Days		I cc.	II cc.	Percentage change	I cc.	II cc.	Percentage change	I cc.	II cc.	Percentage change	I cc.	II cc.	Percentage change	I cc.	II cc.	Percentage change	I °C.	II °C.	Change	I kgm.	II kgm.	Percentage change			
1	4	11	357	356	+0.3	58.5	55.9	+4.7	218	199	+9.6	35.7	31.2	+14.4	139	157	-11.5	22.8	24.7	-7.7	38.6	37.2	+1.4	6.10	6.38	-6.0
2	4	13	285	285	0.0	73.1	72.2	+1.2	193	179	+7.8	49.5	45.1	+9.8	92	106	-13.2	23.6	27.1	-12.9	39.5	36.8	+2.7	3.90	3.95	-1.3
3	3	10	331	331	0.0	70.1	68.8	+1.9	202	191	+5.8	42.8	39.7	+7.8	129	140	-7.9	27.3	29.1	-6.2	36.6	37.0	-0.4	4.72	4.81	-1.9
4	4	10	190	214	-11.2	67.8	75.6	-10.3	122	122	0.0	43.6	43.1	+1.2	68	92	-26.1	24.2	32.5	-25.5	37.0	37.3	-0.3	2.80	2.83	-1.1
5	2	15	447	430	+4.0	69.6	66.6	+4.5	287	293	-2.0	44.7	45.4	-1.5	160	137	+16.8	24.9	21.2	+17.4	39.2	37.0	+2.2	6.42	6.46	-0.6
6	4	9	321	328	-2.1	63.6	63.9	-0.5	205	218	-6.0	40.6	42.5	-4.5	116	110	+5.5	23.0	21.4	+7.5	39.5	37.0	+2.5	5.05	5.13	-1.6
7	3	9	268	298	-10.1	62.3	67.4	-7.6	164	198	-17.2	38.2	44.8	-15.0	104	100	+4.0	24.2	22.6	+7.1	37.0	37.0	0.0	4.30	4.42	-2.7
8	2	16	200	244	-18.0	62.5	71.6	-12.7	129	163	-20.9	40.3	47.8	-15.7	71	81	-12.4	22.2	23.8	-6.7	38.0	37.7	+0.3	3.20	3.41	-6.2
9	2	7	396	483	-18.0	66.4	76.1	-12.8	233	312	-25.3	39.1	49.1	-20.4	163	171	-4.7	27.3	27.0	+1.1	38.0	37.4	+0.6	5.96	6.35	-6.1
10	2	17	487	543	-10.3	87.9	94.8	-7.3	241	327	-26.3	43.5	57.1	-23.8	246	216	+13.9	44.4	37.7	+17.8	37.4	37.6	-0.2	5.54	5.73	-3.3
11	1	23	466	580	-19.7	55.0	65.8	-16.4	266	379	-29.8	31.4	43.0	-27.0	200	201	-0.5	23.6	22.8	+3.5	38.2	37.8	+0.4	8.48	8.81	-3.8

Determination number I made during dehydration, number II after recovery.

The percentage change is obtained by the formula $\frac{100(I-II)}{II}$, so that the change is expressed as the deviation, during dehydration, from the value obtained on recovery.

ANALYSIS OF RESULTS

Blood volume. In table 1 are given the figures for absolute blood volume and blood volume per kilogram of body weight. It will be seen that there is not a constant direction of deviation, during dehydration, from the recovery value; some patients showed no significant alteration, others a marked decrease in the total amount of blood in circulation. The average change proves to be a decrease of about 8 per cent below the value after recovery, but this figure has little significance as an average in view of the dispersion of the results between the extremes of +4 and -20 per cent. No correlation was found between the amount of reduction in circulating blood volume and the severity of dehydration as judged by the usual clinical standards. It is evident from these data that the volume of circulating blood need not necessarily show a diminution even when the subcutaneous tissues are markedly dehydrated (cases 1, 2, 6). For further analysis of the changes in the total volume of circulating fluid, the plasma and cellular components are considered separately.

Plasma volume. Two of the patients showed an apparently significant increase in plasma volume during dehydration (table 1), four of them no significant change, and five a distinct diminution. The work of previous investigators on the dehydration of infants and children (5) and on that of adults in diabetic coma (14) had led us to anticipate a reduction of plasma volume wherever, as in our series, signs of dehydration of the subcutaneous tissues were present. In the light of our own studies, however, we believe that this view must be modified. On comparison of the plasma volume changes with changes in cell volume (table 1), it will be seen that of the first three patients, two of whom showed a significant increase and one a questionable increase in plasma volume during dehydration, all showed a diminution of cell volume. If our results had been the outcome of experimental error in the determination of the blood volume, both plasma and cell volume changes would have been in the same direction, and their divergence is to this extent a confirmation of their validity. It is likely that in patients with dehydration as it is encountered in clinical studies of disease there are in simultaneous operation, in addition to the forces tending to lower the plasma volume on account of the demand for

fluid in other parts of the body, other influences acting in the direction of plasma volume increase. We have shown in experimental animals (15), as Soule, Buckman and Darrow (16) and as Eppinger and Schürmeyer (17) have shown in man, that in the presence of fever the plasma volume tends to be augmented. Many of our patients, as will be seen from Table 1, did have fever at the time the first blood volume

Changes in serum elect

Case number	Total fixed base serum			Chloride serum			Bicarbonate serum			Protein anions serum			Undetermined acids serum		
	I	II	Percentage change	I	II	Percentage change	I	II	Percentage change	I	II	Percentage change	I	II	Percentage change
	cc. N/10 per 100 cc.	cc. N/10 per 100 cc.		cc. N/10 per 100 cc.	cc. N/10 per 100 cc.		cc. N/10 per 100 cc.	cc. N/10 per 100 cc.		cc. N/10 per 100 cc.	cc. N/10 per 100 cc.		cc. N/10 per 100 cc.	cc. N/10 per 100 cc.	
1	137	155	-11.6	83	101	-17.8	21.0	28.8	-27	14.6	13.2	+11	18.4	12.0	+53
2	147	148	-0.7	99	104	-4.8	19.2	26.8	-28						
3	147	156	-5.8	97	106	-8.5	18.6	22.3	-17	18.6	14.6	+27	12.8	13.1	-2
4	145	157	-7.6	99	101	-2.0	16.0	25.7	-38	16.0	12.7	+26	14.0	17.6	-20
5	139	166	-16.3	89	106	-16.0	18.9	25.2	-25	12.7	15.8	-20	18.4	19.0	-3
6	158	157	+0.6	91	102	-10.8	28.4	27.0	+5						
7	140	152	-7.9	95	105	-9.5	13.6	21.0	-35	16.9	12.0	+41	14.5	14.0	+4
8	157	161	-2.5	112	104	+7.7	14.3	25.8	-45						
9	155	165	-6.1	99	105	-5.7	22.4	24.1	-7	17.2	12.0	+43	16.4	23.9	-31
10	162	156	+3.8	97	99	-2.0	19.6	32.0	-39	16.9	12.9	+31	28.5	12.1	+136
11	147	155	-5.2	79	103	-23.3	31.1	28.7	+8	16.2	16.2	0	20.7	7.1	+192

Determination number I made during dehydration, number II after recovery.

The results of determinations are expressed in cubic centimeters of N/10 solution.

The percentage change is obtained by the formula $\frac{100(I-II)}{II}$, so that the change is expressed

determination was made; though it is likewise true that this was not confined to those patients showing an increase of plasma volume during dehydration. The two patients, cases 1 and 2, however, who showed a significant increase, also had fever. The most marked losses of plasma volume were found in patients with temperatures lying between 37.0 and 38.2°C.

Two general methods of visualizing the mechanism of plasma water

exchange have been used in its discussion in the literature. One point of view is exemplified by Schiff (18), who regards the dehydration of the plasma and of the subcutaneous tissues as running closely parallel; water lack affects the one as much as the other. In the second concept, as recently expressed by Gamble (19), the blood is treated as the vehicle of transport of water from sources of supply, such as the

total plasma electrolyte content

fixed base in ation (total plasma)			Chloride in circulation (total plasma)			Bicarbonate in circu- lation (total plasma)			Protein anions in circulation (total plasma)			Undetermined acids in circulation (total plasma)			Undetermined acids in circulation (pro- tein not determined)		
II	Percentage change		I	II	Percentage change	I	II	Percentage change	I	II	Percentage change	I	II	Percentage change	I	II	Percentage change
cc. N/10 per 100 cc.			cc. N/10 per 100 cc.	cc. N/10 per 100 cc.		cc. N/10 per 100 cc.	cc. N/10 per 100 cc.		cc. N/10 per 100 cc.	cc. N/10 per 100 cc.		cc. N/10 per 100 cc.	cc. N/10 per 100 cc.		cc. N/10 per 100 cc.	cc. N/10 per 100 cc.	
309	-2.5	181	201	-10.0	46	57	-19	32	26	+23	40	25	+60				
265	+7.2	191	186	+2.7	37	48	-23								56	31	+81
298	-0.3	196	202	-3.0	38	43	-12	37	28	+32	26	25	+4				
192	-7.8	121	123	-1.6	20	31	-36	20	16	+25	16	22	-27				
486	-17.9	255	311	-18.0	54	74	-27	36	46	-22	54	55	-2				
342	-5.3	187	222	-15.8	58	59	-2								79	61	+30
301	-23.6	156	208	-25.0	22	44	-50	28	24	+17	24	25	-4				
262	-22.5	144	170	-15.3	19	42	-55								40	50	-20
515	-29.9	231	328	-29.6	52	75	-31	40	37	+8	38	75	-49				
510	-23.5	234	324	-27.8	47	105	-55	41	42	-2	68	39	+74				
588	-33.5	210	390	-46.2	83	109	-24	43	61	-34	55	28	+96				

ing dehydration from the value obtained on recovery.

interstitial reservoirs of the subcutaneous tissues, to regions of imperative fluid demand—in cases of diarrhea, the intestinal lumen. This concept envisages the possibility of a normal water content of the blood coexisting with a reduced water content of the tissue reservoirs, and is obviously more in keeping with the results of our own analyses.

The extent of plasma reduction in some of the patients of our series,

amounting to 25 or 30 per cent in some instances, will be of more interest when discussed later in conjunction with parallel changes in plasma electrolytes.

Cell volume. Both increases and decreases were found. While the percentage changes between the two sets of determinations were in many cases quite marked, the absolute changes were not large enough to alter materially our interpretation of parallel plasma volume behavior, even supposing that a replacement of plasma by cells were shown to be a normal physiological exchange. It is not likely that in our patients rapid changes of cell volume were taking place, except in those mentioned as having been transfused. The interval between determinations was long enough, however, to explain changes dependent on more slowly-acting forces, either in the direction of cell volume increase from growth of the blood as a tissue or in the direction of diminution from blood destruction as it occurs in infections.

Electrolyte concentration. The changes in the serum concentration of total fixed base, chloride, bicarbonate, protein anions, and undetermined acid fraction (table 2) are similar to those previously described by other workers. The dominant alteration during dehydration in our series was in the direction of a lowering of concentration of fixed base, chloride, and bicarbonate and an increase in concentration of protein. In a series clinically similar to ours, Hamilton, Kajdi, and Meeker (3) found that 56 per cent showed a subnormal concentration of total base, 40 per cent a low chloride value. The higher figures for total fixed base and for chloride which Hartmann (2) reported may have depended on the fact that his series consisted mainly of patients who were much sicker than ours and who may well have suffered a true anhydremia. Only one of our patients (case 8) showed the increase in chloride concentration at the expense of bicarbonate on which he has laid stress as a common cause of the acidosis accompanying this condition.

Total plasma electrolyte content. We would lay more emphasis, on the other hand, on the data we present concerning the total amount of electrolytes in circulation in the plasma (table 2). The majority in our group showed during dehydration a loss from circulation of total plasma fixed base, and eight out of the eleven a definite diminution in the total plasma chloride. The percentage loss of these sub-

stances is in the great majority of instances larger than the percentage loss of plasma volume, so that if we measure the loss of water from the plasma by the loss of plasma volume, it is clear that what the circulation may lack during dehydration is not water alone but water and electrolytes together; and that the lack of electrolytes may be proportionately more severe. From the standpoint of the therapeutic implications of these studies it is equally clear that one cannot under these circumstances expect to relieve the symptom of dehydration by the administration of water alone or of water in combination with non-electrolyte substances such as glucose.

Composition of fluid leaving the plasma in dehydration. Some idea of the composition of the fluid lost from the plasma may be obtained by calculating the composition of the plasma increase during the period of recovery. The errors involved in such a computation are rather large, and preclude a too strict or literal interpretation of the results; moreover, only when the change in plasma volume from dehydration to recovery is great are these errors sufficiently small to justify such a calculation. For this reason we shall report the composition of the lost plasma fluid only in Case 11, in which the change in plasma volume was greatest and that in cell volume was not appreciable. It is understood also that this involves the precarious assumption that the transition from health to the dehydrated state involves exactly the opposite change from that observed during the interval between dehydration and the second determination of plasma electrolytes. For this patient, the composition of the lost plasma so calculated was:

	cc. N/10 per 100 cc.
Total fixed base.....	174
Chloride.....	159
Bicarbonate.....	23
Protein.....	16
Undetermined acids.....	-24*

* The minus sign means that these anions entered the circulation as the others left it.

It will be observed that this represents a hypertonic solution of electrolytes. The errors of determination referred to deter us, however, from making therapeutic recommendations based on these observations.

Factors determining acidosis. This report includes no examples of

the more severe forms of acidosis. There are certain features, however, which may throw light on the mechanism of development of acidosis under the conditions prevailing. If we arrange the cases in a series in the order of increasing concentration of serum bicarbonate at the time of dehydration, plot the curve of bicarbonate determinations, and on the same coordinates plot the curve of other functions in the same order of cases, the following tendencies are suggested: (1) The patients with low serum bicarbonate are those with subnormal temperature, with a high concentration of serum chloride, and with a relatively large amount of total circulating plasma chloride as shown by the figures for the total plasma chloride during dehydration per kilogram of body weight at that time. (2) There is no parallelism between the tendency to acidosis and the degree of diminution in plasma volume during dehydration (since this diminution may be brought about by a loss of water bound to a neutral mixture of electrolytes), nor is there a demonstrable relationship to the total amount of base lost from the plasma. (3) On the other hand, the patients with low bicarbonate were those showing a low percentage loss of chloride from the plasma; in other words, if the fluid lost from the plasma during dehydration is accompanied by a relatively large amount of base bound to chloride, acidosis is not engendered. This adds confirmation to the now familiar conception (2, 3, 4) that a retention of chloride (or smaller loss relative to base loss) is a frequent factor in the acidosis of diarrhea. (4) It can also be shown where there is a large loss of base *not* bound to chloride, and therefore mainly bound to weak acids, acidosis tends to appear. It must be borne in mind, however, in the interpretation of correlations of this kind, that the method gives no clue as to which of two variables is cause and which effect, or whether any such causal relationship exists at all.

With regard to the therapy of acidosis in the presence of a high serum chloride concentration, one of our patients (case 8) showed rapid and satisfactory recovery, with restoration of serum bicarbonate to normal, after the parenteral administration of salt solution alone and without the use of bicarbonate or any other alkali by mouth. If, as now seems most likely, such chloride retention arises from a functional derangement of the kidney whereby the kidney loses its ability to eliminate chloride bound to the ammonium ^{ion} in the urine,

the restitution of a normal serum electrolyte balance is shown in this instance to be possible when kidney function is restored by whatever means—whether by the injection of salt solution or of some preparation like glucose solution chosen presumably for its diuretic effect (2). Moreover, the experience in this case shows that it is not invariably necessary, in the presence of a chloride acidosis, to resort to alkali therapy provided adequate measures are taken to restore active secretion of urine.

Dehydration of subcutaneous tissues. The degree of dehydration of the subcutaneous tissues, as estimated by the crude quantitative standards described and separated into four grades, shows a suggestive correlation with changes in blood volume in the sense that the milder cases of dehydration suffered the greater diminution of blood volume. In this respect the behavior of plasma volume was more striking. In a series of this size, this may be simply a coincidence; certainly such a correlation was not anticipated, for one might have expected just the opposite—namely, that the patients with most marked lowering of plasma volume would also show the most marked dehydration of the subcutaneous tissues by the usual clinical tests. An explanation for this apparent discrepancy may lie in the factor of the time required for plasma loss to be replaced from intercellular fluid reservoirs, so that when diarrhea has been going on for only a short time the resulting plasma loss has not been met and the plasma has been affected more than the subcutaneous tissues. Of the patients with “slight” or “mild” dehydration, the average duration was 4 days; of those with “moderate” or “severe” dehydration, the average was 15 days. On the other hand, there is evidence from other sources that the response of fluid reservoirs to a need for fluid on the part of the circulating plasma is met in a much shorter time—a matter of minutes. When the body temperature in rabbits is raised, an increase of plasma volume may be detected within an hour (15); and Curtis (20) has shown that a diuresis equivalent to about one-third the entire blood volume may occur within 50 minutes in response to injection of a theophyllin derivative, the fluid presumably being drawn from intercellular reservoirs and conveyed to the kidneys, of course, in the blood. A satisfactory discussion of dehydration of the subcutaneous tissues obviously must await the development of better means of measuring it.

We were not able to demonstrate any close correlation between the severity of "toxicity" and changes in the circulation such as alterations of blood or plasma volume or of the concentration or the total amount of circulating electrolytes. Neither could we show any relationship to serum protein concentration, an increase of which is according to Schiff (19), so closely bound up with the origin of intestinal intoxication. In our series, it by no means followed that the patients showing the most marked lowering of bicarbonate exhibited also the most toxic behavior. As long as the means of obtaining a quantitative measure of the degree of this so-called intoxication are as crude as they are at the present time, the investigation of its origin promises many difficulties.

Plasma proteins. The behavior of the plasma protein component was not uniform: sometimes dehydration was accompanied by a decrease in the total plasma protein (though with no change in serum protein concentration), suggesting that the fluid leaving the circulation under these conditions contained protein in addition to inorganic electrolytes and may possibly have approached the composition of normal plasma, as found by Darrow and Buckman (5); at other times, there was a decrease in total plasma protein during convalescence from dehydration, a possible explanation for which is the starvation to which the patients were subjected in the course of their treatment. The plasma protein changes did not parallel the cell volume changes.

Comparison of blood during dehydration with normal averages. We have already referred to the questionable validity of the assumption that the second set of determinations made in our series represents normal values for that individual. Granted that the patient had been through a serious illness and a period of more or less strenuous treatment, we can only say that he showed at the second analysis no clinical evidence of dehydration, and we have reported these values as found.

Another course is open to us, in that we can compare the values found during dehydration with normal values obtained with the same technique (3, 6), the normal blood volume being calculated on the basis of the body weight. Since this allows us to include patients in whom determinations were made during dehydration but whose death prevented our obtaining a second set for comparison, the series can be

augmented to 19 cases. Without reporting this analysis of data in detail but simply using it as a check on the results already given, we find that it essentially substantiates the statements made in regard to blood and plasma changes in dehydration of this type.

SUMMARY

Eleven infants suffering from dehydration associated with diarrhea were subjected to simultaneous determination of total blood volume and serum electrolyte concentration, both during dehydration and after recovery. In the presence of acute symptoms of dehydration some patients showed no essential change in total blood volume, while others showed a diminution amounting to 20 per cent of the recovery value. When this reduction was marked, it involved principally the plasma fraction, in one instance to the extent of 30 per cent of the recovery figure. There was no close parallelism between the diminution of plasma volume and (1) the degree of dehydration of the subcutaneous tissues as determined by clinical criteria or (2) the severity of toxic symptoms. Most of the patients showed during dehydration a decrease in the serum concentration of total fixed base, chloride, and bicarbonate; but the degree of acidosis, as measured by the serum bicarbonate concentration, could not be correlated with changes in blood or plasma volume, since the patient whose plasma loss was greatest for the whole series had a higher bicarbonate concentration on admission than after recovery. Before treatment had been given, the lowering of the total plasma electrolyte content was sometimes even greater than the loss of plasma water, when measured as percentage deviations from the presumably normal values found after recovery. Protein was often included among the electrolytes lost from the plasma during dehydration, so that the composition of the fluid leaving the circulation was comparable to that of plasma of normal protein and bicarbonate content but concentrated in respect to fixed base and chloride.

The factors governing the development of acidosis associated with dehydration and diarrhea have been briefly discussed, but little emphasis is placed on acidosis as a symptom, since it was not prominent in this series. One patient suffering from a moderate degree of acidosis due to a high concentration of serum chloride was successfully

treated without the use of alkalis and by parenteral injection of sodium chloride solution alone.

The factors influencing the degree of dehydration of the subcutaneous tissues and the severity of toxic manifestations cannot be satisfactorily discussed until more reliable criteria for their quantitative evaluation are available.

As a check on the assumption that the second set of determinations, made after recovery from dehydration but not always at a time of complete clinical recovery, represented normal values for the individual studied, the figures obtained during dehydration were compared with normal values obtained by applying the same technical procedures to healthy infants. This comparison substantiated the results already expressed.

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THE PROBLEM OF SODIUM SALICYLATE EXCRETION IN THE BILE¹

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In the course of studies on the excretion of various chemicals by the liver, it was discovered that sodium salicylate, and the sodium salt of diiodosalicylic acid did not appear in the bile of the rabbit, in determinable quantities, after intravenous administration of 4 cc. of a one per cent solution per kilogram of body weight (1). In view of the differences of opinion regarding the action of sodium salicylate on the biliary system as a cholagogue and as a bactericide (2), it seemed desirable to investigate the excretion of this compound by the human subject. The following is a brief record of experiments on three patients: two of these were cholecystectomized, and subsequently drained bile through their common hepatic duct; while one drained bile through a cholecystostomy.

METHOD

All three patients received by mouth a single dose of 20 mgm. of sodium salicylate per kilogram of body weight. Samples of bile and urine were obtained before administration of the compound, and subsequently the bile and urine were collected at specified intervals for a period of 12, 40 and 64 hours respectively. The following method was employed to determine the presence of sodium salicylate:

Treat 10 cc. bile (or urine) with 90 cc. saturated sodium bicarbonate solution. Extract the alkaline solution with 200 cc. redistilled ethyl ether three times. Discard the ether extract. Neutralize the alkaline solution with 7 N sulphuric acid, using congo red paper as an indicator,

¹ This work has been conducted under the joint auspices of the Otho S. A. Sprague Memorial Institute and the Douglas Smith Foundation for Medical Research.

and add 2 cc. of sulphuric acid in excess. Extract the free salicylic acid three times with ethyl ether, using 200 cc. for each extraction. Test the aqueous extract for the presence of phenols with a drop of ferric chloride. Remove the ether by passing dry air over the dish. Add 1 to 1.5 cc. of saturated sodium bicarbonate solution to the residue and wash quantitatively into a 25 cc. glass stoppered graduated cylinder. Dilute to 20 cc. with distilled water. The salicylic acid is determined in 10 cc. of this liquid by the Millon reaction as modified by Folin and Ciocalteu (3). The color is compared with that produced by 5 mgm. of salicylic acid in 10 cc. of a solution of equivalent alkalinity to the test solution.

CLINICAL OBSERVATIONS

1. E. P., a housewife of 66, was operated on December 3, 1929, to relieve an obstructive jaundice due to stones in the common bile duct. Choledochotomy and cholecystectomy were performed, and a catheter left in place, draining the common hepatic duct. The gallbladder showed gross and microscopic evidence of a chronic cholecystitis. Three mixed gallstones (from 0.5 to 1 cm. in diameter), composed mainly of cholesterol and calcium bilirubinate, were removed from the ductus choledochus, and four more were found in the gallbladder. The stools remained acholic until the 26th of December, 1929. Postoperative convalescence was uneventful. Our experiment began on the 8th of December, 1929. At 8 a.m. samples of bile and urine were collected and 1.2 gram of sodium salicylate was given by mouth. The bile and urine were then collected in hourly samples for 12 hours and tested for their sodium salicylate content by the method outlined above. The flow of bile averaged about 24 cc. per hour (with a minimum of 8 and a maximum of 48 cc.) and totaled 290 cc. for the 12 hours. Sodium salicylate could not be detected in any of these bile samples. The urine totaled 1381 cc. for the same period. This contained a total of 0.584 gram of salicylic acid, corresponding to 0.68 gram of sodium salicylate.

2. I. M., a housewife of 35, was operated on October 18, 1929, to relieve symptoms due to chronic cholecystitis with cholelithiasis. The excised gallbladder showed gross and microscopic evidence of a mild chronic cholecystitis, and contained about 100 mixed gallstones (from 3 to 10 mm. in diameter), of the faceted type, composed mainly of cholesterol and calcium carbonate. Following this operation, the patient became jaundiced and the stools acholic. At a subsequent laparotomy on October 29, 1929, the obstruction was relieved and a T tube was left in place in the common and hepatic ducts. The stools were still acholic when our experiment began on the 30th of November, 1929. At 8:30 p.m. samples of bile and urine were collected, and 1 gram of sodium salicylate was given by mouth.

The bile and urine were collected for a period of 40 hours following the administration of sodium salicylate. Determinations were made on the bile obtained up to the end of the 16th (60 cc.) and the 40th (70 cc.) hours. Neither sample contained any sodium salicylate. The urine collected up to the end of the 34th (870 cc.) and the 40th (185 cc.) hours, following the administration, contained a total of 0.191 gram of salicylic acid, corresponding to 0.222 gram of sodium salicylate.

3. C. W., a housewife of 44, underwent operation on the 17th of December, 1929, because of symptoms and signs indicative of cholecystitis with cholelithiasis. Multiple melanotic tumor nodules greatly enlarged the liver. A cholecystostomy was performed, and the gallbladder emptied of about 150 mixed gallstones (from 2 to 8 mm. in diameter) of the facetted type composed mainly of cholesterol, calcium carbonate and calcium bilirubinate. On the 16th of December, 1929, at 9 p.m., i.e., 15 hours *before* operation, 1.2 gram of sodium salicylate had been given by mouth. The quantity of bile obtained from the gallbladder at operation was insufficient for a test. The bile and urine were collected for a period of 64 hours following the operation. Determinations were made on the three samples of bile obtained at the end of the 16th (178 cc.), 40th (60 cc.) and 64th (57 cc.) hours following the operation. Sodium salicylate could not be detected in any of these samples. The four specimens of urine obtained up to the end of the 12th (229 cc.), 28th (118 cc.), 40th (300 cc.), and 64th (450 cc.) hours following the operation, contained a total of 0.566 gram of salicylic acid, corresponding to 0.655 gram of sodium salicylate.

COMMENT

The principal route for the elimination of sodium salicylate, according to the above observations, is the urinary tract. Sodium salicylate does not appear in the bile in recognizable amounts following oral administration of doses of 20 mgm. per kilogram of body weight. It is, therefore, reasonable to assume, that, whatever beneficial effect may follow the administration of this drug in diseases of the biliary system, is not due to the presence of salicylate in the bile.

SUMMARY

Three patients, two with drainage tubes in the common hepatic duct following cholecystectomy, and one with a cholecystostomy, were given a single dose of sodium salicylate (20 mgm. per kilogram of body weight), and the bile and urine collected for a period of from 12 to 64 hours following the administration. The data obtained indicate that the urinary tract is the principal route of elimination, and that

salicylate does not appear in the bile, following oral administration of a medicinal dose to patients with diseases of the biliary system. These observations are in accord with data obtained in healthy rabbits.

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THE TOLERANCE OF NORMAL SUBJECTS TO LEVULOSE

FACTORS INFLUENCING THE VARIATIONS IN RISE IN BLOOD SUGAR

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MacLean and de Wesselow (1) examined the type of blood sugar curves obtained in normal and diabetic subjects. They suggest that such curves rise very slowly or fail to rise during the second thirty minute period following administration of sugar by mouth, due to stimulation of the glycogen storage mechanism which abstracts the sugar from the blood more quickly than it enters. They believe that this dormant glycogen-forming function of the liver does not begin to act until the sugar concentration approaches 140 mgm. per cent. That the glycogen-storing function is stimulated by hyperglycemic levels is supported by Foster (2). He found that oral administration of glucose, when the concentration of the blood sugar is falling, results in little or no increase in blood sugar. This observation of Foster has been amply confirmed, especially by the work of du Vigneaud and Karr (3) and later by Lennox and Bellinger (4).

That there is a relation between the fasting blood sugar level and its rise above the fasting level following the administration of levulose by mouth seems borne out by this investigation. This relationship is intimately concerned with the mechanism by which the normal subject maintains his fairly constant blood sugar level as recently shown by Jonas and his associates (5), Trimble and Maddock (6) and Sweeney (7). They show that the normal subject maintains a blood sugar almost invariably between 80 and 140 mgm. per cent during such diverse activities as rest, sleep, mild exercise, work, and eating.

Schirokauer (8) was the first investigator who studied the rise in blood sugar following the oral administration of levulose. He found

in normal subjects little or no rise in blood sugar. This work was confirmed by other German investigators (9) (10). MacLean and de Wesselow (1) found levulose to be the only sugar which caused no definite rise in blood sugar in normal subjects. Following up this work, Spence and Brett (11) administered levulose according to body weight to fasting subjects. Using MacLean's blood sugar method and 30 minute samples for two hours, they found the maximum rise in blood sugar to be 12 mgm., and the highest blood sugar obtained to be 112 mgm. per cent. Five normal subjects were studied. Tallerman (12) giving 45 grams of levulose irrespective of the weight to 15 normal subjects concluded that the minimum criteria for an abnormal response to levulose to be: (a) a rise in blood sugar to 135 mgm. per cent, and, (b) an absolute rise of 30 mgm. above the fasting level. Brown (13) giving 20 to 30 grams of levulose to children considers a rise in blood sugar of 30 per cent of the fasting level as evidence of hepatic damage.

PURPOSE OF THIS STUDY

In attempting to evaluate the results of the levulose tolerance test in a group of patients suspected of having impaired liver function (14) it was found that there was no general agreement of the criteria for interpretation of the results. It was then decided to apply the test to a group of normal subjects, large enough to be statistically treated in order to determine:

- (1) The average normal levulose tolerance test.
- (2) Factors influencing the type of curve obtained.
- (3) The changes in tolerance to levulose on repetition of the test.
- (4) Criteria for interpretation of the test.

METHOD

This investigation extended over a period of eleven months from April, 1929. During this time 81 levulose tolerance tests were performed on 49 subjects. Forty of these tests were performed on 32 convalescent patients on the wards of The Third (New York University) Medical Division of Bellevue Hospital. The subjects selected were convalescing from acute infections, who had had normal temperature for ten days and who had been "up patient" for at least three

days. For normal subjects, 17 senior medical students were studied. Forty-one tolerance tests were performed on this group. On repetition of the test the same amount of levulose was given as on the first trial, and an interval of at least 7 days was allowed between tests. Prior to the test all subjects were fasted for 14 hours and reported to the laboratory without breakfast. After a sample of fasting venous blood was obtained, 30, 40, or 50 grams of levulose dissolved in 250 cc. of water were given by mouth. Subjects weighing between 50 and 75 kilos (110 and 165 lbs.) were given 40 grams. Those weighing over 75 kilos were given 50 grams, and those under 50 kilos were given 30 grams. Venous blood samples were obtained at 30, 60, and 120 minute intervals following the administration of the levulose. The protein was precipitated with tungstic acid by the method of Folin and Wu (15) within five minutes of obtaining the sample of blood. Sugar was determined by the method of Folin and Wu (15). A Klett bi-colorimeter was used and all readings were made by the same individual. The levulose used was Pfanstiehl 90 per cent grade which is "practically free from all other sugars, the impurities being calcium levulosate and moisture" (16).

RESULTS

Table 1 gives each of the individual tests arranged according to the fasting blood sugar level. The maximum blood sugar reached was 121 mgm. per cent (Test 66) in a normal subject. The fasting level in this instance was 96 mgm. giving an absolute rise of 25 mgm. in blood sugar. In test 69 a maximum blood sugar of 117 mgm. per cent was obtained with a fasting level of 97 mgm., giving an absolute rise of 20 mgm. In the remaining 79 tests (97.5 per cent) the maximum blood sugar did not exceed 115 mgm. per cent. The maximum rise in blood sugar above the fasting level was 31 mgm. (Test 1) with a fasting level of 69 mgm. per cent. It is noted that rises in blood sugar of more than 25 mgm. above the fasting level occurred in only 6 tests, (Tests 1, 2, 3, 5, 7, 13), and in each instance the fasting blood sugar was 80 mgm. per cent or less.

The average or composite of all 81 tests is given in table 2, and is graphically shown in figure 1. In this curve it will be noted that the rise in blood sugar was 10.1 mgm. occurring at the 30 minute period.

TABLE 1

*The results of 81 levulose tolerance tests on 49 subjects arranged according to the height of the fasting blood sugar level**

Test	Blood sugar				
	Fasting	30 minutes	60 minutes	120 minutes	Rise
	<i>mgm. per cent</i>	<i>mgm. per cent</i>	<i>mgm. per cent</i>	<i>mgm. per cent</i>	<i>mgm. per cent</i>
1	69	100	90	70	31
2	70	81	95	100	30
3	71	95	97	86	26
4	74	96	81	80	12
Composite of tests 1-4	71.0	93.0	90.7	84.0	22.0†
5	76	83	104	80	28
6	76	90	84	79	14
7	76	92	105	88	29
8	77	98	100	96	23
9	77	91	86	84	14
Composite of tests 5-9	76.4	90.8	95.8	85.4	19.4†
Composite of tests 10-25	81.8 ± 1.7	93.5 ± 6.8	92.8 ± 7.1	87.8 ± 9.2	12.0†
Per cent of observations within range of	$\left\{ \begin{array}{l} 1\sigma \\ 2\sigma \\ 3\sigma \end{array} \right.$	$\left\{ \begin{array}{l} 75 \\ 100 \\ 100 \end{array} \right.$	$\left\{ \begin{array}{l} 59 \\ 100 \\ 100 \end{array} \right.$	$\left\{ \begin{array}{l} 68 \\ 87 \\ 100 \end{array} \right.$	$\left\{ \begin{array}{l} 50 \\ 100 \\ 100 \end{array} \right.$
Composite of tests 26-40	87.0 ± 1.4	98.0 ± 6.2	96.2 ± 7.9	86.0 ± 6.7	11.0†
Per cent of observations within range of	$\left\{ \begin{array}{l} 1\sigma \\ 2\sigma \\ 3\sigma \end{array} \right.$	$\left\{ \begin{array}{l} 62 \\ 100 \\ 100 \end{array} \right.$	$\left\{ \begin{array}{l} 78 \\ 100 \\ 100 \end{array} \right.$	$\left\{ \begin{array}{l} 75 \\ 93 \\ 100 \end{array} \right.$	$\left\{ \begin{array}{l} 75 \\ 100 \\ 100 \end{array} \right.$
Composite of tests 41-61	92.0 ± 1.3	101.6 ± 6.3	99.4 ± 9.3	90.6 ± 5.5	9.6†
Per cent of observations within range of	$\left\{ \begin{array}{l} 1\sigma \\ 2\sigma \\ 3\sigma \end{array} \right.$	$\left\{ \begin{array}{l} 71 \\ 100 \\ 100 \end{array} \right.$	$\left\{ \begin{array}{l} 66 \\ 95 \\ 100 \end{array} \right.$	$\left\{ \begin{array}{l} 76 \\ 100 \\ 100 \end{array} \right.$	$\left\{ \begin{array}{l} 76 \\ 95 \\ 100 \end{array} \right.$
Composite of tests 62-75	96.8 ± 1.3	104.8 ± 7.0	99.7 ± 7.3	91.0 ± 5.6	8.0†
Per cent of observations within range of	$\left\{ \begin{array}{l} 1\sigma \\ 2\sigma \\ 3\sigma \end{array} \right.$	$\left\{ \begin{array}{l} 64 \\ 100 \\ 100 \end{array} \right.$	$\left\{ \begin{array}{l} 57 \\ 93 \\ 100 \end{array} \right.$	$\left\{ \begin{array}{l} 85 \\ 93 \\ 100 \end{array} \right.$	$\left\{ \begin{array}{l} 78 \\ 100 \\ 100 \end{array} \right.$

* The individual tests are omitted in the groups of sufficient size to permit of statistical analysis.

† σ = Symbol meaning the Standard Deviation derived from the formula

$$\sigma = \sqrt{\frac{\sum \square^2}{N}}$$

when Σ = Symbol meaning the sum of.

\square = Deviation from the mean.

N = Number of observations.

‡ The rise in the composite curve.

TABLE 1—*Concluded*

Test	Blood sugar				
	Fasting	30 minutes	60 minutes	120 minutes	Rise
	<i>mgm. per cent</i>	<i>mgm. per cent</i>	<i>mgm. per cent</i>	<i>mgm. per cent</i>	<i>mgm. per cent</i>
76	100	103	111	98	11
77	100	100	115	86	15
78	100	109	90	86	9
79	103	109	104	103	6
Composite of tests 76-79	100.8	105.2	100.5	93.2	4.4†
80	105	98	100	93	Nil
81	112	111	102	97	Nil
Composite of tests 80-81	108.5	104.5	101.0	95.0	Nil†

At the 60 minute period the blood sugar is practically unchanged, but at the end of the 120 minutes it has returned to slightly below the fasting level. Statistical methods (17) were applied to these data to see if the sample was of sufficient size. The standard deviation from the mean was determined for each interval. To be statistically true about 66 per cent should fall within the range of the standard deviation, and 95 and 99 per cent of all observations should fall within the range of two and three times the standard deviation respectively. In this series (table 2) the above criteria are fulfilled. The maximum blood sugar allowed by three times the standard deviation is 124 mgm. per cent. Since 97.5 per cent of all tests did not exceed 115 mgm. per cent, and the maximum blood sugar actually obtained was 121 mgm. per cent, it seems safe to assume that blood sugar values reaching 125 mgm. per cent following administration of the specified amounts of levulose to be an abnormal response.

The choosing of convalescent patients as subjects to establish a normal response to a test may be open to criticism, so we included a number of normal subjects. Figure 2 compares the composites of the curves obtained in the normal and convalescent groups. It will be noted that the curves are, for all practical purposes, identical. The greatest deviation from each other is 3 mgm. at the 60 minute period. At this point the probable error of the two composite curves is 1.35

mgm. To be significant there must be a variation of at least three times this error. Therefore the convalescent patients chosen as normal

TABLE 2
Composite curves of various groups of 81 levulose tolerance tests on 49 subjects

Group	Blood sugar				
	Fasting	30 minutes	60 minutes	120 minutes	Rise
	<i>mgm. per cent</i>	<i>mgm. per cent</i>	<i>mgm. per cent</i>	<i>mgm. per cent</i>	<i>mgm. per cent</i>
Entire group	88.7 \pm 8.3	98.8 \pm 8.3	97.2 \pm 8.6	87.6 \pm 7.6	10.1
Per cent of observations within range of	$\left\{ \begin{array}{l} 1\sigma^* \\ 2\sigma \\ 3\sigma \end{array} \right.$	$\left\{ \begin{array}{l} 77 \\ 95 \\ 100 \end{array} \right.$	$\left\{ \begin{array}{l} 68 \\ 96 \\ 100 \end{array} \right.$	$\left\{ \begin{array}{l} 69 \\ 98 \\ 100 \end{array} \right.$	$\left\{ \begin{array}{l} 68 \\ 98 \\ 100 \end{array} \right.$
Normal subjects. 41 tests on 17 subjects	89.8 \pm 8.3	99.4 \pm 8.6	95.7 \pm 9.1	86.6 \pm 6.6	9.6
Per cent of observations within range of	$\left\{ \begin{array}{l} 1\sigma \\ 2\sigma \\ 3\sigma \end{array} \right.$	$\left\{ \begin{array}{l} 78 \\ 97 \\ 100 \end{array} \right.$	$\left\{ \begin{array}{l} 56 \\ 97 \\ 100 \end{array} \right.$	$\left\{ \begin{array}{l} 75 \\ 95 \\ 100 \end{array} \right.$	$\left\{ \begin{array}{l} 68 \\ 100 \\ 100 \end{array} \right.$
Convalescent subjects. 40 tests on 32 subjects	87.6 \pm 7.9	98.3 \pm 7.7	98.8 \pm 7.7	88.6 \pm 8.2	11.1
Per cent of observations within range of	$\left\{ \begin{array}{l} 1\sigma \\ 2\sigma \\ 3\sigma \end{array} \right.$	$\left\{ \begin{array}{l} 77 \\ 95 \\ 100 \end{array} \right.$	$\left\{ \begin{array}{l} 65 \\ 97 \\ 100 \end{array} \right.$	$\left\{ \begin{array}{l} 70 \\ 97 \\ 100 \end{array} \right.$	$\left\{ \begin{array}{l} 72 \\ 97 \\ 100 \end{array} \right.$
First test on 12 normal subjects	91.9 \pm 10.3	100.0 \pm 9.4	95.4 \pm 7.7	89.0 \pm 6.8	8.1
Per cent of observations within range of	$\left\{ \begin{array}{l} 1\sigma \\ 2\sigma \\ 3\sigma \end{array} \right.$	$\left\{ \begin{array}{l} 58 \\ 100 \\ 100 \end{array} \right.$	$\left\{ \begin{array}{l} 67 \\ 100 \\ 100 \end{array} \right.$	$\left\{ \begin{array}{l} 67 \\ 92 \\ 100 \end{array} \right.$	$\left\{ \begin{array}{l} 58 \\ 100 \\ 100 \end{array} \right.$
Second test on 12 normal subjects	92.7 \pm 5.0	100.0 \pm 6.3	93.7 \pm 7.9	86.8 \pm 6.8	7.3
Per cent of observations within range of	$\left\{ \begin{array}{l} 1\sigma \\ 2\sigma \\ 3\sigma \end{array} \right.$	$\left\{ \begin{array}{l} 75 \\ 100 \\ 100 \end{array} \right.$	$\left\{ \begin{array}{l} 67 \\ 92 \\ 100 \end{array} \right.$	$\left\{ \begin{array}{l} 83 \\ 92 \\ 100 \end{array} \right.$	$\left\{ \begin{array}{l} 75 \\ 92 \\ 100 \end{array} \right.$
Third test on 12 normal subjects	89.6 \pm 6.2	100.9 \pm 8.2	97.5 \pm 10.1	85.2 \pm 5.7	11.3
Per cent of observations within range of	$\left\{ \begin{array}{l} 1\sigma \\ 2\sigma \\ 3\sigma \end{array} \right.$	$\left\{ \begin{array}{l} 58 \\ 100 \\ 100 \end{array} \right.$	$\left\{ \begin{array}{l} 75 \\ 100 \\ 100 \end{array} \right.$	$\left\{ \begin{array}{l} 67 \\ 92 \\ 100 \end{array} \right.$	$\left\{ \begin{array}{l} 75 \\ 92 \\ 100 \end{array} \right.$

* Symbol meaning the standard deviation.

subjects gave a response identical to normals, providing the two composite curves are statistically true. The curve of the convalescent

group fulfills these criteria. In the normal group at the 30 minute period not quite 66 per cent of all observations fall within the standard deviation. However, at all other points this is true. Also 95 per cent and 100 per cent fall within two and three times the standard deviation respectively. That these curves are statistically true is even more probable when it is observed that neither curve deviates more than 2 mgm. from the composite of the entire group.

Lennox and Bellinger (4) by triplicate glucose tolerance curves in non-diabetic subjects, in whom the original curve was of the pre-diabetic type, found the average of the second curves lower than the

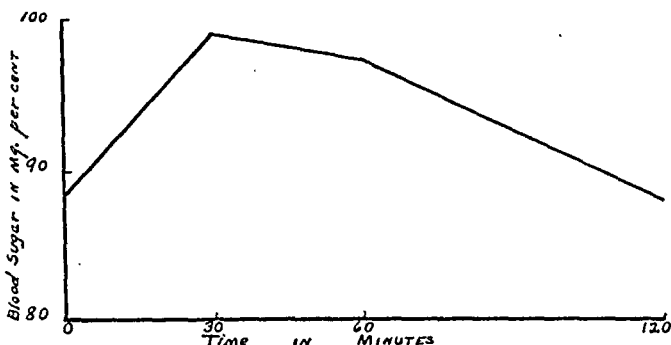


FIG. 1. COMPOSITE OF 81 LEVULOSE TOLERANCE TESTS ON 49 SUBJECTS

first, and the third lower than the second. To see if this apparent improvement in tolerance to glucose was also true for levulose the test was repeated three times on twelve normal subjects at intervals of 7 days or longer. If the tolerance to levulose can be measured by the absolute rise above the fasting level 4 subjects showed a progressive improvement in tolerance to levulose. Three subjects showed a progressive fall in tolerance by the same criterion. If one takes a progressive lowering of the height of the maximum blood sugar as improvement in tolerance, the figures for only one subject show this. By the same criterion one subject showed a progressive impairment in tolerance. By the above methods of analysis there is no evidence of a progressive

improvement in tolerance. If the composite curves for the three groups are examined (fig. 3) we find the three curves almost superimposed. The composite curves of the first tests rises 8.1 mgm., the second rises 7.3 mgm., while the third rises 11.3 mgm. above the fasting level. It is noted, however, that the maximum blood sugar in each of the three composite curves reaches approximately the same level, and variations in the rise are due to changes in the fasting blood sugar level. In all except the fasting period the greatest deviation from the composite curve of all 81 tests is 2.5 mgm. These three curves are statistically

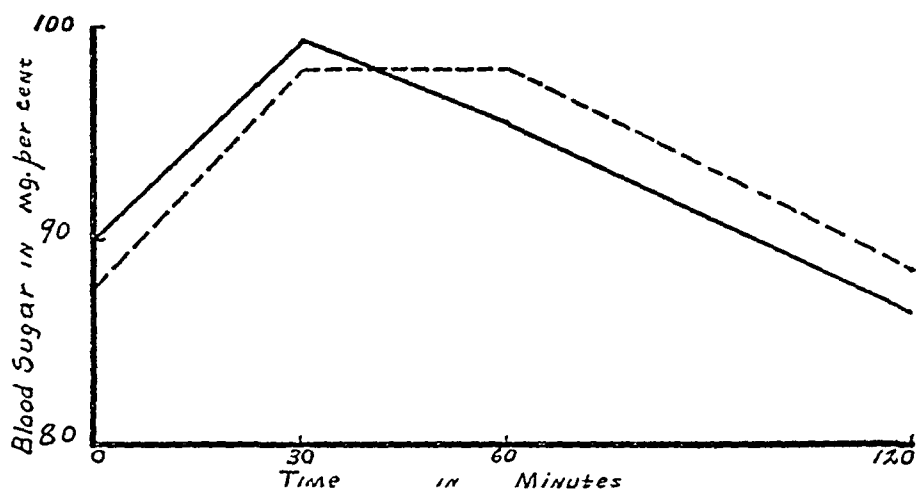


FIG. 2. COMPARISON OF THE COMPOSITE CURVES OBTAINED IN NORMAL AND CONVALESCENT SUBJECTS

Solid line: Normal subjects.

Broken line: Convalescent subjects.

analyzed in table 2. It therefore seems probable that there is no progressive improvement in tolerance to levulose in normal subjects if the tests are repeated at intervals of 7 days or greater.

It soon became apparent that there are great variations in the rise in blood sugar above the fasting level, from individual to individual, and to a less extent in the same individual. It has been previously noted in this paper that rises of more than 25 mgm. above the fasting level occurred only when the fasting blood sugar was 80 mgm. per cent or less. It was also noted that the differences in rise in blood sugar in the

composite curves in the subjects on whom triplicate curves were performed were due mainly to changes in the fasting blood sugar level, rather than to the height to which the curve rose. It appears, therefore, that after administration of levulose the blood sugar rises from whatever fasting value it may have, high or low, toward a constant "ceiling," and then falls as sugar storage is accelerated. Following this idea our data have been tabulated according to the height of the fasting blood sugar, and composites made for each group of 5 mgm. from 75 to 104 mgm. per cent. Fasting values below 75 mgm. were in-

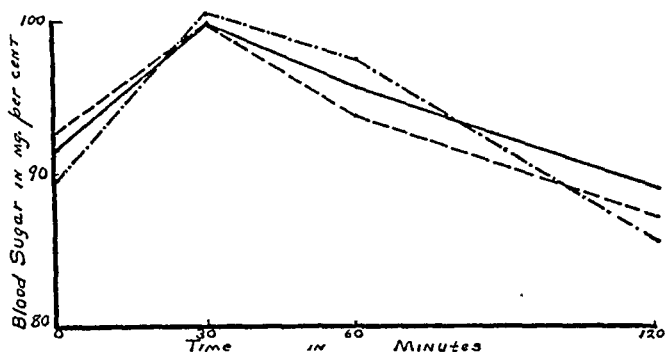


FIG. 3. TRIPPLICATE LEVULOSE TOLERANCE CURVES ON 12 NORMAL SUBJECTS

Solid line: Composite curve of the first tests.

Broken line: Composite curve of the second tests.

Dash-dot-dash line: Composite curve of the third tests.

cluded in one group, as were those of 105 mgm. or over. Figure 4 gives the various composite curves obtained. It is clear that the higher the fasting blood sugar level the smaller is the rise in blood sugar above that level. This relationship is better shown in the ogive correlation in figure 5. With a fasting level below 75 mgm. note that the rise is 22 mgm., while in the group with a fasting level above 104 mgm. note that the rise is nil. In the groups between 80 and 99 mgm. this relationship is statistically true, and therefore probably not the result of random sampling. However, for the two ex-

treme groups of high and low fasting values the number of curves is too small to permit conclusions to be drawn. It may be calculated that 400 tolerance tests would be required to produce a minimum of ten cases in each of these groups if the distribution of the fasting blood sugar values remained as in this group of 81 tests.

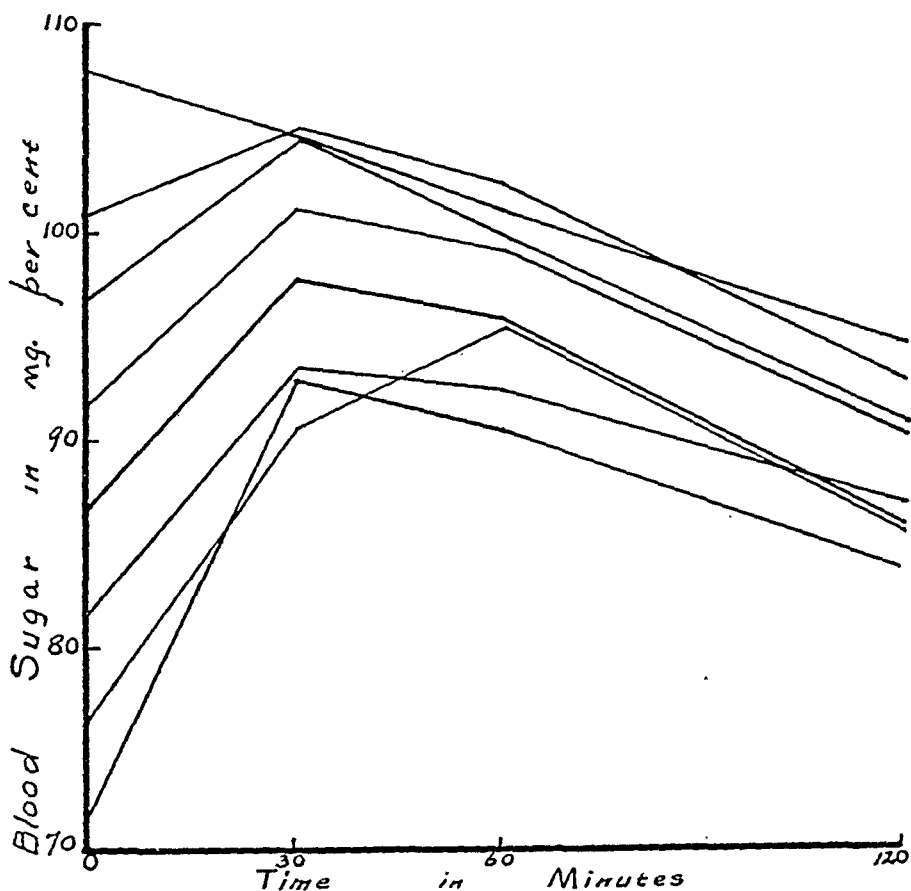


FIG. 4. RELATION OF THE FASTING BLOOD SUGAR LEVEL TO THE RISE IN BLOOD SUGAR FOLLOWING ORAL ADMINISTRATION OF LEVULOSE

Since extremely low and extremely high fasting levels cannot be obtained spontaneously with sufficient frequency, we extended this series by raising the fasting blood sugar to slightly over 100 mgm. per cent by giving glucose prior to the administration of levulose. In a

preliminary test on a normal subject using 20 grams of glucose the blood sugar rose from a fasting level of 89 mgm. to 109 mgm. in 30 minutes, and then progressively fell to 81 mgm. in two hours (Curve 2, fig. 6). Ten days later this same individual was tested to 40 grams of glucose when the blood sugar rose to 104 mgm. in 15 minutes, to 138 in 30 minutes, and then progressively fell, reaching 85 mgm. at the two hour period (Curve 1, fig. 6). It therefore seemed that 20 grams of glucose administered by mouth would raise the blood sugar sufficiently

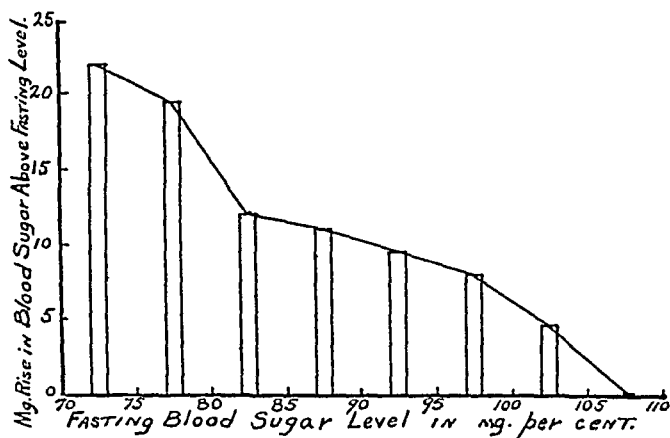


FIG. 5. OGIVE CORRELATION OF THE FASTING BLOOD SUGAR LEVEL TO THE RISE IN BLOOD SUGAR FOLLOWING ORAL ADMINISTRATION OF LEVULOSE

to obtain a "fasting" blood sugar of over 100 mgm. per cent within 30 minutes. Ten normal subjects volunteered for this study. In each of these subjects the administration of the preliminary dose of 20 grams of glucose preceded the administration of the levulose by 30 minutes. In the composite curve of the results obtained (Curve 4, fig. 6) the blood sugar rose from a fasting level of 84.4 mgm. to slightly over 100 mgm. per cent in thirty minutes. At this time 40 mgm. of levulose were given. There was in the composite curve no rise following the administration of the levulose, but a progressive fall very much like the

curve obtained when only 20 grams of glucose (Curve 2, fig. 6) was given alone. The individual curves are given in table 3. In 4 sub-

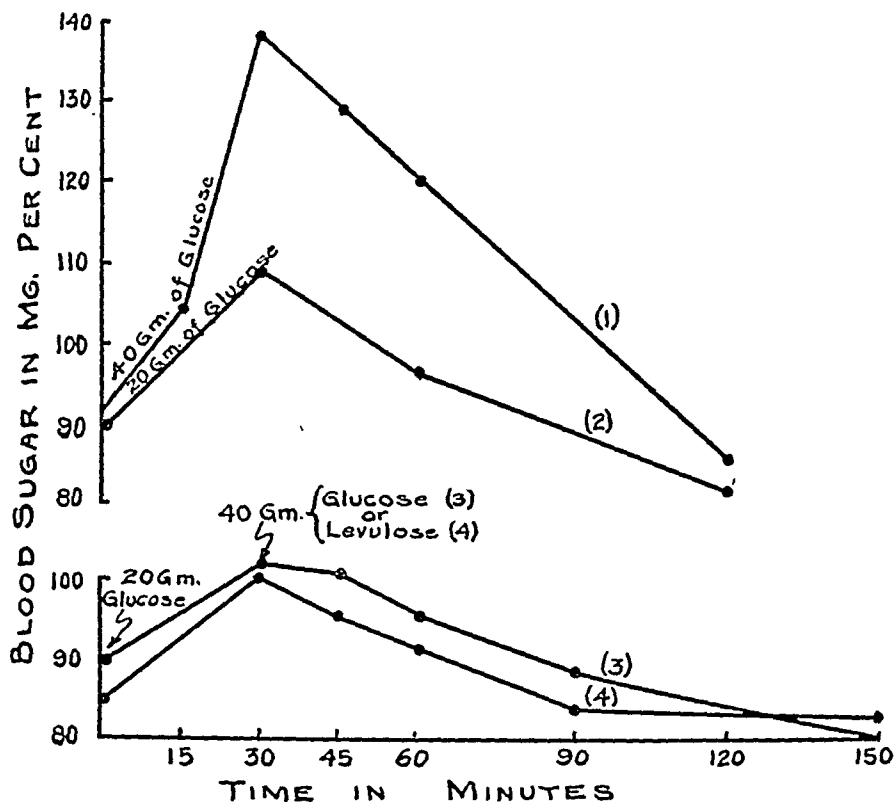


FIG. 6. CURVES ILLUSTRATING THE EFFECT OF ARTIFICIAL ELEVATION OF THE BLOOD SUGAR LEVEL PRIOR TO THE LEVULOSE OF GLUCOSE TOLERANCE TESTS

Curve 1. Blood sugar curve following oral administration of 40 grams of glucose.

Curve 2. Blood sugar curve following oral administration of 20 grams of glucose.

Curve 3. Composite curve of the effect of administering 20 grams of glucose 30 minutes prior to the glucose tolerance test in 10 normal subjects.

Curve 4. Composite curve of the effect of administering 20 grams of glucose 30 minutes prior to the levulose tolerance test in 10 normal subjects.

jects there was a slight rise at the 45 minute period above the 30 minute period. In these subjects, however, the 30 minute blood sugar did not exceed the average for the group, thus agreeing very closely

TABLE 3

Effect of administration of 20 grams of glucose thirty minutes prior to levulose tolerance test

Subject	Blood sugar							
	Fasting		30 minutes		45 minutes	60 minutes	90 minutes	150 minutes
	<i>mgm. per cent</i>		<i>mgm. per cent</i>		<i>mgm. per cent</i>	<i>mgm. per cent</i>	<i>mgm. per cent</i>	<i>mgm. per cent</i>
50	87	20 grams glucose	94	40 grams levulose	88	100	82	87
51	88		116		98	96	102	102
52	87		89		93	82	80	86
53	78		98		93	80	80	76
54	80		95		96	87	76	79
55	75		100		87	82	80	80
56	86		103		84	86	80	74
57	94		109		100	92	86	80
58	88		100		106	100	91	82
59	81		98		100	97	80	81
Composite...	84.4		100.2		94.5	91.2	83.7	82.4

TABLE 4

Effect of administration of 20 grams of glucose thirty minutes prior to glucose tolerance test

Subject	Blood sugar							
	Fasting		30 minutes		45 minutes	60 minutes	90 minutes	150 minutes
	<i>mgm. per cent</i>		<i>mgm. per cent</i>		<i>mgm. per cent</i>	<i>mgm. per cent</i>	<i>mgm. per cent</i>	<i>mgm. per cent</i>
50	101	20 grams glucose	103	40 grams glucose	105	93	71	90
51	95		111		105	100	93	83
52	86		114		104	96	92	77
53	90		114		112	105	84	83
54	83		90		103	95	94	58
55	83		88		90	94	94	75
56	89		89		81	80	81	80
57	96		109		100	90	82	71
58	94		99		101	100	92	68
59	84		102		100	98	90	86
Composite...	90.1		101.9		100.1	95.1	87.3	79.8

with the results obtained in those tests having a "natural" fasting blood sugar of 95 to 104 mgm. The individuals having a blood sugar at the thirty minute period above the average for the group invariably

showed a lowering at the 45 minute period, agreeing very closely with the composite curve of those individuals having a "natural" fasting blood sugar of 105 mgm. or over. These results were so consistent that it was decided to substitute the same amount of glucose for the levulose administered at the thirty minute period. The same subjects again volunteered and a glucose tolerance test was preceded by thirty minutes by 20 grams of glucose. It was our purpose to show that if glucose was administered to subjects with a high normal blood sugar that a hyperglycemia would result. Curve 3 in figure 6 shows that such was not the case, but the curve was practically identical with the case when levulose was administered. Table 4 gives the individual curves. It is noted, however, that when glucose was given the fall during the first portion of the curve was possibly not as rapid as when levulose was given.

DISCUSSION

The factor or factors influencing the type of blood sugar curve obtained by various sugars, notably glucose, levulose, and galactose have been variously explained. Folin and Berglund (18) consider absorption of sugar from the blood by the tissues rather than glycogen formation the immediate reason the administered sugar fails to accumulate in the blood. The slight rise in blood sugar when levulose is administered is explained by the fact that the tissues are practically free from levulose, and relatively "saturated" with glucose. This hypothesis has been questioned by Foster (2), Reinhold and Karr (19) and by Bodansky (20) who show that there is a greater rise in blood sugar with galactose than with glucose. Reinhold and Karr (19) believe that the ability of various carbohydrates to produce hyperglycemia varies directly with their rate of absorption, and inversely with their ability to form glycogen and their ease of being oxidized. Levulose is absorbed slowly but, being a good glycogen former and readily oxidized, causes only a slight rise in blood sugar. Cori and Cori (21) have shown in rats that levulose is absorbed more slowly than glucose and that galactose is absorbed more rapidly than glucose after oral administration. In four hours time approximately 16 per cent of the glucose absorbed is deposited in the liver as glycogen (21) while 40 per cent of the levulose and 5 per cent of the galactose absorbed are deposited in the liver as glycogen.

The facts presented in this paper seem to add additional information upon the type of blood sugar curve obtained by glucose and levulose, and by inference the explanation for the type of curve obtained with galactose. We may assume first, that there is an excitatory ceiling or level of blood sugar in normal subjects between 95 and 110 mgm. per cent at which the sugar storage mechanism is stimulated. We may further assume, since crystalline insulin itself shows a latent period when injected intravenously (22), that an interval of 10 to 15 minutes elapses after this excitatory ceiling is reached before the sugar storage mechanism begins to effect a significant fall in blood sugar. In this view sugar administered per os would drive the blood sugar above the excitatory ceiling because of the continued absorption during the latent period. Those sugars which are most rapidly absorbed (galactose) would overshoot the ceiling considerably and the blood sugar curve would not start to fall until a level of about 160 mgm. per cent had been reached: sugars of slower absorption (glucose) would overshoot the ceiling less (140 mgm. per cent) and slowly absorbed sugar (levulose) would overshoot it but little (115 mgm. per cent). MacLean and de Wesselow (1) believe the level of blood sugar at which the carbohydrate mechanism is stimulated to be about 140 mgm. following administration of glucose. We have shown that if the blood sugar is raised by a preliminary dose of 20 grams of glucose to 100 mgm. per cent, a second dose of 40 grams is followed by an immediate fall, and not a rise to about 140 mgm. as would be expected by the explanation of MacLean and de Wesselow. This result agrees with our interpretation that the sugar storage mechanism is stimulated at a blood sugar level of 95 to 110 mgm. per cent, and was therefore active when the second dose of sugar was given. The high blood sugar level obtained at the thirty minute period when 40 grams of glucose alone was given (fig. 6, Curve I) is the result of an overshooting of the excitatory ceiling during the latent period in consequence of its absorption during the 15 to 30 minute period.

This hypothesis also explains why the rise in the blood sugar above the fasting level following the administration of levulose varies inversely with the height of the fasting blood sugar level. In an individual having a very low fasting level the rise must of necessity be comparatively great before a fall occurs since the storage mechanism

probably remains dormant until a level of 95 to 110 mgm. has been reached. This hypothesis seems to explain the progressive fall in blood sugar in the group having a fasting level of 105 mgm. or more.

SUMMARY AND CONCLUSIONS

Ninety-one levulose tolerance tests and 12 glucose tolerance tests have been performed on normal subjects under a variety of conditions. Results have been tabulated according to the fasting blood sugar level with the following conclusions:

1. In normal subjects the maximum blood sugar following administration of 30 to 50 grams (based on body weight) of levulose by mouth rarely exceeds 115 mgm. per cent.

2. A rise in blood sugar to 125 mgm. per cent or over should be considered an abnormal response to the oral administration of levulose.

3. In the group of convalescent subjects included in this study the levulose tolerance was the same as in the normal subjects studied.

4. Triplicate levulose tolerance tests performed on normal subjects 7 or more days apart show no significant variation in their blood sugar curves.

5. In normal subjects the rise in blood sugar above the fasting level following the oral administration of levulose varies inversely with the height of the fasting blood sugar level.

6. It seems probable that there is a level of blood sugar varying from 95 to 110 mgm. per cent, which when exceeded stimulates the sugar storage mechanism to clear the blood rapidly of its excess sugar. A period of 10 to 15 minutes elapses after this excitatory ceiling is reached before the sugar storage mechanism begins to cause a significant fall in blood sugar. The rise in blood sugar above this level depends upon the rapidity of absorption of the sugar administered. The rapidity with which the blood sugar falls from its peak probably depends upon the glycogen forming ability of the sugar used.

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NUCLEOTIDE THERAPY IN AGRANULOCYTOSIS

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In recent years many reports (1 through 14) have appeared of a condition first described by Schwarz (15) and called by Schultz (16) agranulocytic angina. This is characterized by fever, by intense prostration, by a sore throat, sometimes almost gangrenous in appearance, by large purpuric areas on the body which frequently become necrotic, and by a marked leukopenia, especially of the polymorphonuclear cells. The course is usually acute and the mortality is so high that when a patient recovers it is sufficiently significant to merit a report.

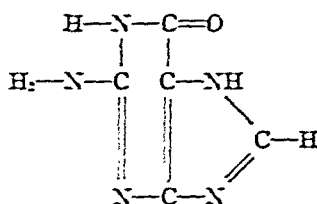
No definite causative agent has as yet been found. Moreover, some skepticism exists as to whether agranulocytic angina is a clinical entity (14). Blumer (17) describes agranulocytosis, the outstanding feature of the condition, in other infectious diseases and Wilson (18), and McCord (19) point out its occurrence in arsenic and benzene poisoning. On the Second Medical Division of Bellevue Hospital a considerable depression of the granulocytes was found in some of the patients with pneumonia and tuberculosis.

Roberts and Kracke (20) believe that there is a definite disease entity known as agranulocytosis and base their opinion on a case which showed a marked marrow deficiency before any other symptom or sign occurred. Their conclusion does not necessarily follow because blood cell response usually precedes other symptoms and signs in most conditions. For instance a marked "shift to the left" of the polynuclears in an infectious condition is usually present from 24 to 48 hours before other symptoms and signs are seen (21). The present evidence makes it probable that agranulocytosis is due to a depression of the production of granulocytes or of their delivery into the circulation because of an

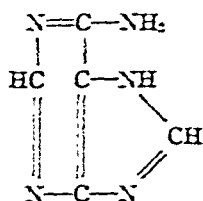
inherent failure of the particular organism in the course of a toxemia rather than because of any definite toxic agent.

Agranulocytosis has been treated principally by transfusions and roentgen therapy. Since in this condition the other blood elements are usually not affected (22), this disease should lend itself to treatment by the stimulation of the polymorphonuclear elements by nucleotides such as adenine sulfate and guanine hydrochloride. Doan, Zerfas, Warren and Ames (23) and Reznikoŭ (24) have shown that the nucleotides, when given intravenously, cause a marked increase in the polymorphonuclear cells in rabbits without any effect on the temperature or on blood cells other than the granulocytes.

Guanine or 2-amino-6-oxypurine has the empirical formula $C_5H_5N_3O$ and has the structural formula



Adenine has the empirical formula $C_5H_5N_5$ with the structural formula



These two materials, obtained from the Eastman Kodak Company, are both prepared by the hydrolysis of yeast nucleic acid. This is hydrolyzed with 10 per cent sulfuric acid. The solution is made alkaline, which causes a precipitation of the crude guanine. The adenine remains in the alkaline solution. The crude guanine is dissolved in hot hydrochloric acid. Upon cooling the crystals of guanine hydrochloride precipitate out. The alkaline filtrate containing the adenine is made just acid. To this solution copper sulfate is added which causes the formation of an insoluble adenine cuprous compound. This compound is broken up with hydrogen sulfide which results in the precipitation of the copper. The filtrate, after the removal of the cuprous sulfide, is evaporated to dryness. This residue is dissolved in hot sulfuric acid and upon cooling the adenine sulfate crystallizes out.

In the patients to be described 0.5 gram of the nucleotide was given intravenously in about 20 cc. of physiological saline. The nucleotide was boiled in the saline. Certain preparations of nucleotides are insoluble in saline. In such cases 10 per cent HCl was added drop by drop during the boiling until a homogeneous suspension was formed. If introduced into the vein slowly a little discomfort was felt with the administration of the acidulated product and none with the soluble nucleotide.

The following results were obtained with the patients suffering from agranulocytic angina.

*Patient I.*¹ Young male physician at the City Hospital, New York City, developed a sore throat, headache, chills, cough, was nauseated and vomited, became delirious and showed a large slough on his buttock. The other salient features of his condition are given in table 1.

The patient's throat cleared up completely. He was extremely weak and the slough on his buttock healed slowly. He was discharged in good condition to a convalescent home on March 19, 1928. In June, 1929 he had an attack of acute tonsillitis at home. His temperature was 101-102 for about 2 days and his physician reported a normal blood count. On June 25th his count was 7400 white blood cells, 65 per cent polymorphonuclears. His tonsils were removed in July, 1929 with no untoward results. He was in perfect health until May, 1930 when he developed some apical abscesses of two teeth. Vincent's spirillum was recovered and upon extraction of the teeth in June another attack of agranulocytosis was precipitated. The details of this attack are not available but the essential features are the following: After two transfusions of 500 cc. the patient's condition became worse and the blood count decreased to 500 white blood cells no polymorphonuclears being seen in the entire slide. His physician, Dr. Jacobs began injection of adenine sulphate, giving 0.5 gram in 25 cc. of saline very day. In addition he continued daily transfusions. Six hours after the injection of the first dose of adenine sulphate, the white blood cell count began to rise, polymorphonuclears appeared and the patient's general condition began to improve. He was discharged in a month and has been in good health since.

*Patient II.*² A middle aged woman had an unhealed cyst at the lower end of her spine. During her stay in the hospital a blood count showed 3400 white blood cells and no polymorphonuclears were seen in at least 300 white cells. Generalized ultraviolet treatment was instituted and she improved. One month later her total white blood cell count was 7000 of which 51 per cent were polymorphonu-

¹ I am indebted to Drs. Jacobs, Lisa, Coyne and the other members of the City Hospital Staff for permission to report this case.

² I wish to thank Dr. Herbert Bergamini and the staff of the Reconstruction Hospital for their help and permission to quote this case.

TABLE 3
Progress of Patient III treated during course of illness with nucleotide

Date (1929)	Time	Temperature	White blood cells	Percentage polymorphonuclears	Treatment
		^{°F.}			
June 18.....	8:00 a.m.	104	2,200	0	
June 19.....		103-104			
	8:00 a.m.	102			Leucocyte extract (Squibb) 10 cc.
June 20.....	4:00 p.m.	104.2	1,600	2	Leucocyte extract (Squibb) 10 cc.
	12:00 p.m.	104.6			Leucocyte extract (Squibb) 10 cc.
	4:00 a.m.	104			Leucocyte extract (Squibb) 10 cc.
June 21.....	12:00 m.	104	2,000	0	Leucocyte extract (Squibb) 10 cc.
	8:00 p.m.	104.2			Adenine sulfate
June 22.....	4:00 a.m.	104			Adenine sulfate
	4:00 p.m.	104	1,800	0	Transfusion 500 cc.
June 23.....	4:00 a.m.	103.8			Intravenous glucose 500 cc. of 20 per cent solution
June 24.....	12:00 m.	104	2,300	3	Transfusion 250 cc.
June 25.....	8:00 a.m.	103			Adenine sulfate
	8:00 a.m.	100	4,800	14	
June 26.....	9:45 a.m.	100			Adenine sulfate
	12:00 m.	100			Transfusion 250 cc.
	10:30 p.m.	101			Adenine sulfate
June 27.....	8:00 a.m.	99	8,200	31	Adenine sulfate
	8:00 a.m.	102	18,000	42	
June 28.....	12:00 m.	100.5			
	4:00 p.m.	102.5			
	8:00 a.m.	104	21,000	59	
June 29.....	12:00 m.	101			
	4:00 p.m.	103.6			
	8:00 a.m.	103.8			
June 30.....	12:00 m.	100			
	8:00 p.m.	105.2			
July 1.....		100	17,000	68	
July 2.....		98.6	15,600	72	
July 3.....		98.6	13,200	74	
July 4.....		98.6	12,400	69	
July 5.....		98.6	11,600	64	

morphonucleurs just before death. This agonal rise should not be confused with nucleotide action. It seems probable, however, that nucleotide, pushed vigorously, can stimulate the bone marrow in pneumonia and its use may have an important place in the treatment of the disease when leukopenia is present.

The blood count of one agranulocytic patient with pulmonary tuberculosis gave no response to nucleotide therapy.

COMMENTS

The patients reported here do not represent the most desirable type of experimental material. They were extremely sick and every possibly helpful therapeutic measure was indicated. For example the 3 patients who recovered from agranulocytic angina received blood transfusions in addition to nucleotide. However, according to most authorities (1-14), the mortality rate in agranulocytic angina is just as high in patients who receive transfusions as in untreated patients. Moreover, the case of the patient who died from streptococcus hemolyticus septicemia illustrates that a transfusion, especially a large amount, markedly decreases the polymorphonuclear cells in the peripheral blood. If blood is to be transfused it should be given in small amounts, not over 250-cc. at a time, because of this depressing effect.

Friedemann (13) reports good results with x-ray therapy. Six of ten patients recovered after such treatment, whereas only 2 of 24 untreated patients recovered previously in his series.

More complicated chemical products have been used for similar purposes, especially in pneumonia (25 and 26) and as long ago as 1897 Ames and Huntley (27) showed that nucleic acid from yeast will increase the polymorphonuclear count. The demonstration of Buell and Perkins (28) that adenine occurs normally in the blood makes its use more rational than the more complex compound.

At present 0.5 gram of the nucleotide is given twice in 24 hours. Some response is seen within 6 hours after the injection and usually the increase in granulocytes is marked after the second dose.

CONCLUSIONS

1. Nucleotides given intravenously have the power of raising the peripheral polymorphonuclear count in certain agranulocytic conditions.

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STUDIES IN CONGESTIVE HEART FAILURE

VI. THE EFFECT OF OVERWORK AND OTHER FACTORS ON THE POTASSIUM CONTENT OF THE CARDIAC MUSCLE¹

BY J. ALFED CALHOUN, GLENN E. CULLEN, GURNEY CLARKE AND
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(Received for publication June 12, 1930)

In one of the previous studies of this series it was shown that the cardiac and skeletal muscles of patients dying of congestive heart failure were abnormally poor in potassium, Harrison, Pilcher and Ewing (1930). The loss of potassium from skeletal muscle appeared to be related to edema, as muscles which were not edematous contained normal amounts of potassium. Pieces of skeletal muscles removed during life contained less potassium when the patients were edematous than when they were edema free. It appears therefore that the presence of edema is an important cause of the loss of potassium in skeletal muscle. The present study was undertaken in an attempt to determine the cause of the loss of potassium from the cardiac muscle.

METHOD

Tissues were obtained at the postmortem table. Pieces of ventricular muscle weighing 5 to 20 grams were freed of fat, weighed and dried to constant weight at 105 to 110°C. One to two decigrams of the dried muscle were digested with nitric acid as described by Van Slyke (1924) for chloride determinations. The nitric acid was then driven off by evaporation, and the residue was freed of ammonia by boiling with excess sodium hydroxide. The mixture was then neutralized with sulphuric acid. Potassium was precipitated as the cobalti-

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nitrate and determined in the Van Slyke-Neill apparatus as described by Kramer and Gittleman (1926). A detailed description of the technique was given by Harrison, Pilcher and Ewing.

Duplicate analyses were always made and in many instances quadruplicate determinations were done. The values in the tables are averages of these duplicate and quadruplicate determinations. The tissues were obtained from patients dying of cardiac failure and, to serve as controls, from patients dying of various other disorders. Tissues of normal individuals were of course not available as controls, and we have even been unable to obtain tissues from individuals dying accidentally who were in good health previously. Hence, subjects having no disease of the thoracic organs have been designated as controls. (One individual with severe anemia, and one patient with morphinism have been omitted from the "control" group for reasons which will be mentioned later.)

As the study progressed it was found that patients with acute pulmonary disorders showed similar changes and these were consequently grouped together.

The subjects with cardiac disease were classified according to the type of congestive failure which they presented. Most of them had both systemic and pulmonary congestion but one patient had congestion of the lungs only, two patients had systemic without pulmonary congestion and one subject had cardiac disease without congestive failure. All except one of the subjects with cardiac disease had cardiac hypertrophy and dilatation of varying degree. The chief etiological factors were (a) rheumatic endocarditis, one case; (b) syphilitic aortitis, one case; (c) syphilitic myocarditis, one case; (d) hypertension, four cases; (e) coronary arteriosclerosis and angina pectoris, two cases; (f) asthmatic bronchitis, one case; and (g) tuberculous pericarditis, one case.

RESULTS

The data for the "control" subjects are shown in table 1. These values are in fairly close agreement with those obtained for human subjects by Lematte, Boinot and Kahane (1928), and by Norn (1929). The three "control" subjects all died of chronic diseases and it is therefore possible that the normal values for man are slightly higher.

TABLE I
The potassium content of cardiac muscle of subjects without cardiac or pulmonary disease

Initials	Autopsy number	Chief diagnosis	Right ventricle				Left ventricle				Congestion of lung	Congestion of liver	Edema	Chief pathological findings	Remarks
			Solids	Potassium in dry muscle	Potassium in wet muscle	Solids	Potassium in dry muscle	Potassium in wet muscle	Solids	Potassium in dry muscle	Potassium in wet muscle				
E. A.	V-30-5	Lymphosarcoma	18.6	1.41	0.262	22.4	1.29	0.289			0	0	±	Enlarged glands	Edema of right leg from lymphatic obstruction. None elsewhere
R. O.	V-29-17	Brain tumor	20.5	1.30	0.267	23.0	1.19	0.274			0	0	0	Spongioblastoma	
J. S.	V-30-32	Carcinoma of esophagus	21.0	1.21	0.254	19.9	1.47	0.292			0	0	0	Perforation of esophagus	

nitrate and determined in the Van Slyke-Neill apparatus as described by Kramer and Gittleman (1926). A detailed description of the technique was given by Harrison, Pilcher and Ewing.

Duplicate analyses were always made and in many instances quadruplicate determinations were done. The values in the tables are averages of these duplicate and quadruplicate determinations. The tissues were obtained from patients dying of cardiac failure and, to serve as controls, from patients dying of various other disorders. Tissues of normal individuals were of course not available as controls, and we have even been unable to obtain tissues from individuals dying accidentally who were in good health previously. Hence, subjects having no disease of the thoracic organs have been designated as controls. (One individual with severe anemia, and one patient with morphinism have been omitted from the "control" group for reasons which will be mentioned later.)

As the study progressed it was found that patients with acute pulmonary disorders showed similar changes and these were consequently grouped together.

The subjects with cardiac disease were classified according to the type of congestive failure which they presented. Most of them had both systemic and pulmonary congestion but one patient had congestion of the lungs only, two patients had systemic without pulmonary congestion and one subject had cardiac disease without congestive failure. All except one of the subjects with cardiac disease had cardiac hypertrophy and dilatation of varying degree. The chief etiological factors were (a) rheumatic endocarditis, one case; (b) syphilitic aortitis, one case; (c) syphilitic myocarditis, one case; (d) hypertension, four cases; (e) coronary arteriosclerosis and angina pectoris, two cases; (f) asthmatic bronchitis, one case; and (g) tuberculous pericarditis, one case.

RESULTS

The data for the "control" subjects are shown in table 1. These values are in fairly close agreement with those obtained for human subjects by Lematte, Boinot and Kahane (1928), and by Norn (1929). The three "control" subjects all died of chronic diseases and it is therefore possible that the normal values for man are slightly higher.

TABLE 2
The potassium content of cardiac muscle of subjects with acute diseases of lungs (and with no cardiac disease)

Initials	Autopsy number	Chief diagnosis	Right ventricle						Left ventricle						Congestion of lungs	Congestion of liver	Edema	Chief pathological findings	Remarks
			Solids			Potassium in			Solids			Potassium in							
			Per cent	dry muscle	wet muscle	Per cent	dry muscle	wet muscle	Per cent	dry muscle	wet muscle	Per cent	dry muscle	wet muscle					
E. H.	V-29-111	Eclampsia	17.5	0.80	0.140	19.7	1.67	0.328				0	0	0		0	0	Massive collapse both lungs. Bronchopneumonia. Focal necrosis of liver	Hypertrophy of left ventricle. Right ventricle dilated
J. D.	V-30-24	Carcinoma of gallbladder	20.4	0.78	0.159	20.6	1.23	0.254				0	0	0		0	0	Massive collapse both lungs. Carcinoma of gall bladder and liver	Postoperative. Right ventricle dilated
W. S.	V-29-119	Ruptured appendix	18.6	1.07	0.198	19.6	1.52	0.298				0	0	0		0	0	Massive collapse left lung. Peritonitis	Postoperative. Questionable slight dilatation of left ventricle
R. J.	V-30-33	Lobar pneumonia	22.4	0.89	0.199	19.1	1.37	0.262				0	0	0		0	0	Lobar pneumonia bilateral. Acute pericarditis	Right ventricle dilated

Normal dogs usually have about 1.4 per cent potassium in the dried cardiac muscle and 0.3 per cent potassium in the wet cardiac muscle, (Calhoun, Cullen and Harrison (1930)). In any case it is safe to conclude that the normal potassium content of wet cardiac muscle is 0.3 per cent or slightly less and that the normal solids content is 19 to 23 per cent.

The term "normal potassium content" when employed in the remainder of this paper is used to designate the average (table 4) values in the "control" subjects. It is of some interest to note that in each instance the right ventricle contained a little less potassium in the wet muscle than did the left.

In table 2 are shown the values in four patients with acute and more or less extensive disease of the lungs. One of them had pneumonia; three had massive pulmonary collapse—one unilateral and two bilateral. Their left ventricles contained amounts of potassium comparable to those found in the "controls." Their right ventricles, however, were extremely low in potassium. As compared with the average (table 4) value for the "controls" the potassium content of the dry right ventricular muscle of the patient with unilateral pulmonary collapse was minus 18 per cent. A similar comparison reveals minus 39 per cent and minus 40 per cent for the two patients with bilateral collapse and minus 32 per cent for the patient with lobar pneumonia. It is interesting that the degree of diminution was least in the patient who had the least extensive pulmonary involvement. All of these subjects had normal hearts at autopsy except for slight dilatation of the right ventricle.

Values for subjects with cardiac disease are shown in table 3. In only one subject (S. C.) was there disease of the heart without any systemic or pulmonary congestion. This subject, who had hypertension and angina pectoris, died suddenly of coronary occlusion. A fresh infarct of the left ventricle and old infarcts of the interventricular septum were found. The left ventricle was somewhat hypertrophied, the right ventricle slightly dilated. The tissues taken for analysis were from the normal portions of the myocardium. Each ventricle contained a little less than the normal amount of potassium in the dry muscle.

Two subjects in this group were classified as having only right ventricular failure. (For the sake of clarity it should be stated here that the term "failure of a ventricle" as used in this paper, denotes a condition in which that ventricle is dilated and the tissues from which it receives blood are congested or edematous.) In neither of them were the lungs congested at autopsy. Both had some edema and some congestion of the liver. One of these subjects (W. H.) had hypertrophy and dilatation of the right ventricle only. His right ventricle was abnormally poor in potassium and his normal-sized left ventricle contained more than did those of the "control" subjects. J. M. (table 3) had hypertension, pleural effusion, partial collapse of both lungs and adhesive pericarditis. His left ventricle was hypertrophied but not dilated. It contained in the dry muscle 18 per cent less than the average amount of potassium. His right ventricle was hypertrophied and dilated at autopsy and it contained 30 per cent less than the average normal amount of potassium. As has been stated, he had right but not left ventricular failure, i.e., systemic without pulmonary congestion.

Seven individuals had failure of both sides of the heart. In all of them (table 3) the potassium content of both ventricles was much decreased in both the wet and dry tissues. The etiology of the cardiac disease seemed to be of no particular importance. The changes found in the patient with rheumatic endocarditis (D. McK.) and in the two subjects with syphilitic disease (J. F. and J. J.) were of the same order of magnitude as those observed in the two patients with "essential" hypertension (F. H. and J. R.), in the subject with hypertension secondary to chronic glomerulonephritis (J. B.), and in the individual who had hypertrophy and dilatation without obvious etiology (E. R.). The type of rhythm also made no difference, and no correlation with electrocardiographic changes was found. Consequently, data concerning the cardiac rhythm are omitted from the tables.

The findings in C. W. are of especial interest. This man had *concretio cordis* with a pericardium more than one half centimeter thick. The heart seemed only slightly enlarged by physical examination and x-ray. He had congestion of the liver and some edema.

TABLE 3

The potassium content of cardiac muscle of subjects with cardiac disease

Initials	Autopsy number	Chief diagnosis	Right ventricle						Left ventricle			Congestion of lung	Congestion of liver	Edema	Chief pathological findings	Remarks
			Solids	Potassium in dry muscle	Potassium in wet muscle	Solids	Potassium in dry muscle	Potassium in wet muscle	Solids	Potassium in dry muscle	Potassium in wet muscle					
S. C.	V-29-115	Coronary occlusion	26.2	1.10	0.288	20.5	1.21	0.249				0	0	0	Hypertrophy left ventricle, dilatation right ventricle, occlusion left descending artery	Never had congestive failure. Sudden death
W. H.	V-29-112	Asthmatic bronchitis	21.4	1.09	0.233	19.4	1.60	0.310				0	++	++	Chronic bronchitis, tuberculosis, emphysema, hypertrophy and dilatation of right ventricle only	Right ventricular failure
J. M.	V-30-31	Pleurisy with effusion, hypertension	17.6	0.92	0.161	21.3	1.07	0.227				0	=	+	Pleural effusion, adhesive pericarditis, hypertrophy both ventricles, dilatation right ventricle, collapse of both lungs	Right ventricular failure (slight)
A. McC.	V-30-20	Angina pectoris. Hypertension	19.9	1.03	0.206	20.3	0.91	0.184				++	0	0	Coronary arteriosclerosis, myocardial fibrosis, hypertrophy and dilatation both ventricles	Left ventricular failure
J. B.	V-30-21	Chronic glomerulonephritis, hypertension	18.5	0.94	0.174	19.9	1.02	0.203				++	++	++	Chronic glomerulonephritis, hypertrophy and dilatation both ventricles	Failure of both ventricles
J. F.	V-30-17	Syphilis	19.6	1.10	0.216	19.5	1.02	0.200				--	--	--	Hypertrophy and dilatation of both ventricles, syphilitic myocarditis and aortitis	Failure of both ventricles
J. J.	V-29-125	Syphilitic aortic insufficiency	18.7	0.95	0.177	20.4	0.98	0.199				--	--	--	Hypertrophy and dilatation of both ventricles, syphilitic aortitis	Failure of both ventricles
F. H.	V-29-10	Hypertension	18.6	0.95	0.177	19.4	0.83	0.162				--	--	--	Hypertrophy and dilatation of both ventricles	Failure of both ventricles
J. R.	V-29-7	Hypertension	19.9	0.85	0.148	19.9	1.10	0.221				--	--	--	Hypertrophy and dilatation of both ventricles, myocardial fibrosis	Failure of both ventricles
E. R.	V-30-35	Cardiac hypertrophy	17.5	0.97	0.170	18.6	1.07	0.199				--	--	--	Hypertrophy and dilatation both ventricles, thrombophlebitis, septicemia.	Failure of both ventricles
D. McK.	V-30-36	Rheumatic endocarditis	16.1	0.79	0.127	18.6	1.07	0.180				--	--	--	Aortic stenosis and insufficiency, hypertrophy, and dilatation both ventricles, pericardial effusion, ascites.	Failure of both ventricles
C. W.		<i>Concretio cordis</i>	22.2	1.17	0.260	22.1	1.64	0.361				0	--	--	Obliterative pericarditis without mediastinitis, pericardium $\frac{1}{4}$ cm. thick	Atrophy of myocardium, massive collapse, bilateral-post-operative

Two subjects in this group were classified as having only right ventricular failure. (For the sake of clarity it should be stated here that the term "failure of a ventricle" as used in this paper, denotes a condition in which that ventricle is dilated and the tissues from which it receives blood are congested or edematous.) In neither of them were the lungs congested at autopsy. Both had some edema and some congestion of the liver. One of these subjects (W. H.) had hypertrophy and dilatation of the right ventricle only. His right ventricle was abnormally poor in potassium and his normal-sized left ventricle contained more than did those of the "control" subjects. J. M. (table 3) had hypertension, pleural effusion, partial collapse of both lungs and adhesive pericarditis. His left ventricle was hypertrophied but not dilated. It contained in the dry muscle 18 per cent less than the average amount of potassium. His right ventricle was hypertrophied and dilated at autopsy and it contained 30 per cent less than the average normal amount of potassium. As has been stated, he had right but not left ventricular failure, i.e., systemic without pulmonary congestion.

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The findings in C. W. are of especial interest. This man had *concretio cordis* with a pericardium more than one half centimeter thick. The heart seemed only slightly enlarged by physical examination and x-ray. He had congestion of the liver and some edema.

The heart sounds were very faint, the pulse weak and the pulse pressure low. The cardiac pulsations as viewed by the fluoroscope were very small. Following an operation at which an unsuccessful attempt to resect the pericardium was made he developed pulmonary collapse and died. At autopsy the myocardium was thin and atrophic. This man suffered from pericardial insufficiency, not from myocardial failure. His difficulties were due to inability of the heart to dilate. His atrophic left ventricle was unusually rich in potassium, his right ventricle contained a little less than the normal amount in the dry muscle. It is possible that his right ventricle would also have con-

TABLE 4
Average values for potassium and solids content of cardiac muscle

Group number	Type of case	Number of cases	Right ventricle			Left ventricle		
			Solids	Potassium in dry muscle	Potassium in wet muscle	Solids	Potassium in dry muscle	Potassium in wet muscle
			<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	No cardiac or pulmonary disease	3	20.0	1.31	0.261	21.8	1.32	0.285
2	No cardiac disease. Acute pulmonary disease	4	19.7	0.89	0.174	19.8	1.45	0.286
3	Cardiac hypertrophy without congestive failure	1	26.2	1.10	0.288	20.5	1.21	0.249
4	Right ventricular failure only	2	19.5	1.00	0.197	20.3	1.34	0.269
5	Left ventricular failure only	1	19.9	1.03	0.206	20.3	0.91	0.184
6	Failure of both ventricles	7	18.4	0.94	0.171	19.5	0.99	0.195
7	Myocardial atrophy	1	22.2	1.17	0.260	22.1	1.64	0.361

tained increased amounts of potassium had he not developed pulmonary collapse, which, as has been shown above, is often followed by loss of potassium from the right ventricle. (Further clinical data and hemodynamic studies on this man are being reported elsewhere by Burwell and Strayhorn (1930).)

For convenience in comparison the average values in the various groups are presented in table 4. Despite the relatively small number of cases, it is believed that these values are significant because of the uniformity of the changes observed.

DISCUSSION

The average values for the content of solids of the heart muscle of the subjects with cardiac failure were slightly lower than those of the control individuals but the difference was small and the two groups overlap in this respect. It, of course, follows that the water content of the heart muscle in the two groups is not dissimilar, and therefore, that the variation in potassium content cannot be explained on the basis of edema. It is clear that cardiac muscle, unlike skeletal muscle, does not become markedly edematous.

From the data in tables 3 and 4 it appears that when myocardial insufficiency results in pulmonary congestion the potassium content of the left ventricle is diminished and that when myocardial insufficiency results in hepatic congestion and systemic edema the potassium content of the right ventricle is decreased. The question immediately arises as to which is cause and which is effect. Does the loss of potassium precede or follow failure of the corresponding ventricle?

An answer to this question is probably to be found in Table 2. There is reason to believe that the flow of blood through atelectatic lung tissue is diminished whether the atelectasis be due to collapse or consolidation. Consequently, in such conditions the vascular channel through which the right ventricle pumps blood is narrowed with a corresponding rise in the pressure against which this chamber must work. Accentuation of the pulmonic second sound and slight dilatation of the right ventricle in such cases are well recognized phenomena. There can be little doubt that in such extensive and sudden disease of the lungs the right ventricle is overworked.

The fact that our patients with acute pulmonary disorders showed such a marked diminution in the potassium of their right ventricles seems to indicate that overwork or "strain" can cause loss of potassium from the cardiac muscle. The patients did not have any of the peripheral signs of right ventricular failure and hence loss of potassium is not due to congestive failure. Therefore, since diminished potassium content was invariably present in failing ventricles it is likely that this chemical change may have been a contributing cause of such failure. A ventricle which has failed is always low in potassium but a ventricle which is low in potassium need not necessarily have failed.

Dilatation of a ventricle was associated in every instance with a diminution in the potassium content and every ventricle which had a decreased potassium content was more or less dilated. The studies of Starling and his co-workers have shown that the normal immediate response to increase in work is dilatation. It seems probable, therefore, that loss of potassium and dilatation occur coincidentally and that both are due to overwork.

Most of the hypertrophied ventricles were also dilated and all ventricles showing both phenomena had diminished potassium content. In three subjects, however, hypertrophy of the left ventricle was present without dilatation or failure. In two of these (S. C. and J. M. (table 3)) there was a slight diminution in the potassium content of the dry muscle. The third subject, E. H., (table 2) had eclampsia and increased blood pressure. The left ventricle was unusually rich in potassium, containing about fifteen per cent more than those of the "normal" controls. Hypertrophy of a ventricle, therefore, may be associated with decreased potassium content, but not necessarily so.

Atrophy of the myocardium was associated with a tendency toward a high potassium content in one case, (C. W., table 3). Although this man had systemic congestion he did not have myocardial failure. His heart muscle was atrophic and the cavities were abnormally small. He suffered from pericardial failure, his heart was unable to dilate because of its dense fibrous coating.

In a previous study (Harrison, Pilcher and Ewing (1930)) it has been pointed out that diminished potassium content may be related in some way to "cardiac fatigue." From the results of the present study it appears that overwork is the cause of loss of potassium, and that this loss may be one of the causes of cardiac fatigue and eventual congestive failure. We do not believe that potassium is the so element concerned. It is likely that the composition of the heart in patients with cardiac failure is altered in other respects. Investigations are in progress along these lines.

SUMMARY OF RESULTS

1. The water content of the ventricular muscles of subjects dying of congestive heart failure was not significantly increased.
2. Patients dying with acute and extensive disease of the lungs

(i.e., pneumonia and massive collapse) had diminished potassium content of the right ventricle, but not of the left ventricle.

3. When myocardial insufficiency results in pulmonary congestion the potassium content of the left ventricle is diminished.

4. When myocardial insufficiency results in hepatic and systemic edema the potassium content of the right ventricle is decreased.

5. If both systemic and pulmonary congestion were present both ventricles were poor in potassium.

6. The cardiac potassium was not diminished in a subject with *concretio cordis* and myocardial atrophy.

7. The dilated ventricles were poor in potassium; the ventricles which were hypertrophied but not dilated showed variable results.

CONCLUSIONS

1. Edema is not the cause of the loss of cardiac potassium.

2. It is believed that overwork causes loss of potassium from heart muscle and that this loss is one of the predisposing factors to cardiac fatigue and failure.

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contract against a weight of several hundred grams. The strength of the current and the number of stimuli were varied in the different experiments in such a way as to keep the contractions as rapid as possible without the development of local tetany. When local tetany did supervene stimulation was stopped for a few minutes and then was begun again at a slower rate. The number of twitches per minute varied between thirty and two hundred.

After this stimulation had gone on for several hours the animal was killed and samples were taken from the two gastrocnemius muscles. Determinations of potassium content were carried out according to the technique which has already been described (Harrison, Pilcher and Ewing).

RESULTS

In six of the ten experiments the muscle from the stimulated leg contained somewhat less solids than did that from the normal leg. In one instance the reverse was true. In the remaining three experiments, the water contents of the two legs were practically the same. Average values of water content were 25.1 per cent in the normal muscle and 24.3 per cent in the stimulated muscle. The difference is too small to be significant. In this connection it is of interest to note that Barcroft and Kato (1915) found increased rate of passage of water into actively contracting muscles.

The duration of stimulation was eight hours or less in four experiments. In one of these the potassium content of the dry tissue from the stimulated leg was slightly greater than that from the normal leg. In another, the values were practically the same. In the remaining two instances the dried tissues from the stimulated leg contained less potassium than those from the normal extremities.

The duration of stimulation was eleven hours or more in the other six experiments and in all of these the dried muscle of the stimulated leg was poorer in potassium, the average diminution as compared with the control specimen from the same animal being approximately 20 per cent. Average values for the entire series of experiments were 1.46 per cent for the normal legs and 1.25 per cent for the stimulated legs. The potassium content of the wet muscle from the stimulated leg was higher than that of the control leg in one instance and lower in

nine instances. The single exception occurred in one of the experiments of short duration. Average values for all the experiments were 0.362 per cent potassium for the normal legs and 0.304 per cent for the stimulated legs. If only the six experiments of longer duration be considered the differences are somewhat greater, the averages being 0.371 per cent and 0.289 per cent.

TABLE 1

The effect of stimulation of the sciatic nerve on the solids and potassium content of the gastrocnemius muscle

Experiment number	Duration of stimulation	Solids		Potassium content of dry muscle		Potassium content of wet muscle	
		Normal leg	Stimulated leg	Normal leg	Stimulated leg	Normal leg	Stimulated leg
	<i>hours</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
P ₁	6	24.5	21.6	1.60	1.60	0.391	0.345
P ₂	8	23.7	22.7	1.38	1.26	0.327	0.284
P ₃	6	26.6	27.8	1.29	1.37	0.343	0.381
P ₇	5	26.7	25.7	1.33	1.14	0.354	0.292
P ₈	12	24.2	24.0	1.52	1.29	0.369	0.310
P ₉	11	26.2	25.2	1.38	1.16	0.362	0.293
P ₁₁	12	23.8	22.6	1.64	1.33	0.390	0.300
P ₁₂	13	25.2	25.3	1.48	1.15	0.372	0.291
P ₁₃	12	25.5	25.7	1.45	1.17	0.370	0.301
P ₁₄	13	24.4	22.4	1.50	1.08	0.365	0.242
Average.....		25.1	24.3	1.46	1.25	0.362	0.304

DISCUSSION

These experiments seem to show clearly that overwork of a skeletal muscle, if of sufficient degree and duration, is likely to be followed by a decrease in its potassium content. The loss of potassium is probably to be ascribed to an increase in the hydron concentration since Höber (1929) observed in vitro that increase in hydron concentration increased the diffusion of potassium from the muscle, and Hawk and Bergheim (1926) state that acidosis causes increased excretion of potassium.

There is indirect evidence (Harrison, and Pilcher (1930)) that edema may cause tissue oxygen lack. It is well known that lack of oxygen tends to cause local acidosis. It has been shown that edematous

THE NUMBER OF FORMED ELEMENTS IN THE URINARY SEDIMENT OF PATIENTS SUFFERING FROM HEART DISEASE, WITH PARTICULAR REFERENCE TO THE STATE OF HEART FAILURE

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It is well known that casts, red blood cells and white blood cells may be present in the urinary sediment of patients suffering from heart failure. In the manner that specimens of urine are usually obtained it is not possible to gain an accurate notion of the numbers of cells which are present, since variations in salt concentration and reaction of the urine may result in partial or complete disappearance of the formed elements of the urine. Addis (1) recommended a procedure which has for its object the secretion by the kidneys of urine of such high specific gravity and acidity that the integrity of the formed elements of the urine is maintained. By this means the number of elements in urinary sediment formed in a 12-hour period may be estimated. Addis (2) found that the number of casts passed in a 12-hour period varied in normal individuals between 0 and 4,270, the average being 1,040; the number of red blood cells between 0 and 425,000, the average being 65,700; the number of white blood and epithelial cells between 32,400 and 1,835,000, the average being 322,500. The casts were hyaline; granular casts were not observed.

The present study is concerned with the estimation of the number of formed elements in the urines of patients suffering from chronic heart disease, especially of the so-called arteriosclerotic type, more particularly with reference to the state of heart failure of the congestive type.

The patients were at rest in bed and were in water equilibrium. Observations were made in patients suffering from the congestive type of heart failure, and again in the same patients after the signs and symptoms of heart failure had disappeared. In others, observa-

Case number	Hospital number	Age and sex	State with reference to heart failure	Diagnosis*	
				Etiological	Anatomical
1	7106	40 M.	During failure (++++) [†] Recovered	Arteriosclerosis	Cardiac hypertrophy, mitral insufficiency, chronic myocarditis
2	7054	47 M.	During failure (+++) Recovered	Arteriosclerosis	Cardiac hypertrophy, mitral insufficiency, chronic myocarditis
3	7255	74 M.	During failure (+) Recovered	Arteriosclerosis	Cardiac hypertrophy
4	7318	68 M.	During failure (++) Recovered	Arteriosclerosis	Cardiac hypertrophy, chronic myocarditis, VPL [§]
5	7108	60 M.	During failure (+) Recovered	Arteriosclerosis	Chronic myocarditis
6	6784	40 F.	During failure (++++) During failure (+++) During failure (++) Recovered	Hypertension, 180/130	Cardiac hypertrophy, mitral insufficiency, aortic roughening, VPL
7	4692	58 M.	During failure (++) Recovered	Arteriosclerosis, hypertension	Cardiac hypertrophy, mitral insufficiency, chronic myocarditis
8	7091	34 M.	During failure (++++) Recovered	Rheumatic fever	Cardiac hypertrophy, mitral stenosis, mitral insufficiency, aortic roughening
9	7325	34 M.	During failure (+) Recovered	Rheumatic fever	Cardiac hypertrophy, mitral stenosis
10	7223	27 M.	During failure (++) Recovered	Rheumatic fever	Cardiac hypertrophy, mitral insufficiency and stenosis, aortic roughening
11	7311	40 M.	During failure (++++) Recovered	Rheumatic fever	Cardiac hypertrophy, mitral insufficiency and stenosis

* The diagnoses in this table as well as in tables 2, 4 and 5 conform to the nomenclature for cardiac di.

[†] The degree of heart failure is indicated by + signs in this table as well as in tables 2 and 4.

[‡] In this table as well as in table 2 and 4, + and 0 indicate the presence or absence of the sign.

[§] VPL = left ventricular preponderance in this table as well as in tables 2, 4 and 5.

C.H.F. = heart failure of the congestive type in this table as well as in tables 2, 4 and 5.

I-HB = incomplete heart block in this table as well as in tables 2, 4 and 5.

g and after recovery from heart failure

Signs of heart failure†							Number of attacks of heart failure	Medication effective in relief of heart failure	Urea clearance	Phenolsulphthalein excretion in 2 hours	Sediment test (Addis)				
Edema	Hydrothorax	Rales	Cyanosis	Ascites	Liver	Dyspnea					Casts			Red blood cells, number in 12 hours	White blood cells, number in 12 hours
											Number in 12 hours	Hyaline	Granular		
									<i>per cent of normal</i>	<i>per cent</i>		<i>per cent</i>	<i>per cent</i>		
+	+	+	+	+	+	+	1	Limitation of fluids and rest in bed	93	74	0 91,400	25	75	292,500 1,050,000	1,023,750 2,520,000
+	+	+	+	+	+	+	1	Digitalis	64 55	54 59	23,800 65,920	67 67	33	98,000 3,090,000	70,000 834,000
+	0	+	0	0	+	0	1	Digitalis	62	36	24,232 9,240	100 100		2,514,625 165,000	1,514,625 577,500
+	0	0	0	+	0	0	1	Limitation of fluids			0 22,110	50	50	994,000 904,500	745,500 603,000
±	0	+	0	0	0	0	1	Limitation of fluids	58	53	33,200 59,400	66 75	33 25	300,000 674,000	800,000 1,215,000
+	+	+	+	+	+	+	1	Digitalis	97	48	4,890 10,080	99 100	1	208,000 156,800	1,300,000 1,232,000
±	+	0	+	±	+	0		Digitalis	107	72	11,220	100		105,000	1,056,000
0	0	0	0	0	0	0		Digitalis and theocalcin	160	62	141,600	100		480,000	2,257,500
+	0	+	+	0	+	+	2	Digitalis	95 77	54 67	72,000 16,400	100 100		99,450 157,000	5,670,000 2,201,000
+	0	+	0	+	+	+	3	Digitalis	29 32	53	3,905 19,740	50 100	50	585,750 705,000	461,500 1,938,750
±	0	0	0	+	0	±	1	Digitalis	113	61	151,060 0	66	33	1,365,000 630,000	2,047,500 1,050,000
0	0	+	+	0	+	+	8	Digitalis			0 12,600			1,402,500 1,462,500	627,500 1,912,500
±	0	+	+	+	+	+	3	Digitalis			31,350 62,150	50 33	50 66	1,140,000 706,250	1,425,000 706,000

TABLE 2

The number of formed elements found in the urine during heart failure of the congestive type

Case number	Hospital number	Age and sex	State with reference to heart failure	Diagnosis			Signs of heart failure							Urea clearance	Phenolsulphonphthalein excretion in 12 hours	Sediment test (Addis)					
				Etiological	Anatomical	Physiological	Edema	Hydrothorax	Rales	Cyanosis	Ascites	Liver	Dyspnea			Number of attacks of heart failure	per cent of normal	per cent	Number in 12 hours	Hyaline	Granular
12	7078	48 F.	During failure (+++++)	Arteriosclerosis, hypertension	Cardiac hypertrophy, aortic roughening	Normal rhythm, C.H.F.*	+	+	+	+	+	+	+	1			36,080	100	100	451,000	6,478,000
13	7184	68 M.	During failure (+++++)	Arteriosclerosis, hypertension	Cardiac hypertrophy, chronic myocarditis	Auricular flutter, C.H.F.	+	0	0	+	+	+	+	1			144,375	90	10	393,750	656,250
14	7110	65 M.	During failure (+)	Arteriosclerosis	Cardiac hypertrophy, chronic myocarditis, VPL	Normal rhythm, APC, VPC,* C.H.F.	+	0	0	0	0	0	0	1	14	37	17,928	100		540,000	864,000
15	6911	54 F.	During failure (+++++)	Arteriosclerosis, rheumatic fever	Cardiac hypertrophy, mitral insufficiency, aortic roughening, VPL	Normal rhythm, VPC, C.H.F.	+	+	+	+	+	+	+	1	69	50	280,000	100		183,750	3,675,000
16	7085	52 M.	During failure (+++++)	Rheumatic fever	Cardiac hypertrophy, mitral insufficiency, mitral stenosis chronic myocarditis, VPR*	Auricular fibrillation, C.H.F.	+	+	+	+	+	0	+	1			463,740	90	10	2,478,000	17,877,000
17	7323	27 M.	During failure (++++)	Rheumatic fever	Cardiac hypertrophy, mitral stenosis and insufficiency, cardiac dilatation	Auricular fibrillation, C.H.F.	+	0	+	+	+	+	+	9			0			1,402,500	627,500
18	7222	36 M.	During failure (+++++)	Syphilis	Cardiac hypertrophy, aortic insufficiency	Normal rhythm, C.H.F.	+	+	+	+	+	+	+	1	70	89	29,925		100	72,210	4,132,250

* C.H.F. = heart failure of the congestive type
 VPR = right ventricular preponderance
 APC = auricular premature contractions
 VPC = ventricular premature contractions

in this table as well as in table 4.

TABLE 3
Summary of the numbers of formed elements found in the urine of cardiac patients

Type	Number of patients	Formed elements	Lowest number	Highest number	Average number	Distribution of patients
Patients who had not suffered from cardiac failure	7	Casts	0	80,000	17,792	4* N† (2 = 0†, 2 H†); 3 > † N
		Red blood cells	22,250	428,000	162,531	7 N (2 A, † 5 H)
		White blood cells	96,000	1,788,000	809,555	7 N (2 A, 5 H)
Patients after recovery from heart failure	16	Casts	0	141,600	34,600	4 N (4 = 0); 12 > N
		Red blood cells	165,000	3,090,000	917,515	4 N (4 H); 12 > N
		White blood cells	304,000	2,520,000	1,151,187	13 N (2 A, 11 H); 3 > N
Patients during heart failure	18	Casts	0	463,740	66,485	6 N (4 = 0, 2 H); 12 > N
		Red blood cells	72,210	3,090,000	834,754	9 N (1 A, 8 H); 9 > N
		White blood cells	70,000	17,877,000	3,089,302	13 N (1 A, 12 H); 5 > N

* Refers to number of patients.

† N = normal, A = average, H = highest normal, 0 none.

‡ > N = greater than normal.

TABLE 2

The number of formed elements found in the urine during heart failure of the congestive type

Case number	Hospital number	Age and sex	State with reference to heart failure	Diagnosis		Signs of heart failure							Urea clearance	Phenolsulphthalein excretion in 12 hours	Sediment test (Addis)						
				Etiological	Anatomical	Physiological	Edema	Hydrothorax	Rales	Cyanosis	Ascites	Liver			Dyspnea	Number of attacks of heart failure	Number in 12 hours	Hyaline	Granular	Red blood cells, number in 12 hours	White blood cells, number in 12 hours
12	7078	48 F.	During failure (+ + + + +)	Arteriosclerosis, hypertension	Cardiac hypertrophy, aortic roughening	Normal rhythm, C.H.F.*	+	+	+	+	+	+	+	1			36,080	100	100	451,000	6,478,000
13	7184	68 M.	During failure (+ + + + +)	Arteriosclerosis, hypertension	Cardiac hypertrophy, chronic myocarditis	Auricular flutter, C.H.F.	+	0	0	+	+	+	+	1			144,375	90	10	393,750	656,250
14	7110	65 M.	During failure (+)	Arteriosclerosis	Cardiac hypertrophy, chronic myocarditis, VPL	Normal rhythm, APC, VPC,* C.H.F.	+	0	0	0	0	0	0	1	14	37	17,928	100		540,000	864,000
15	6911	54 F.	During failure (+ + + +)	Arteriosclerosis, rheumatic fever	Cardiac hypertrophy, mitral insufficiency, aortic roughening, VPL	Normal rhythm, VPC, C.H.F.	+	+	+	+	+	+	+	1	69	50	280,000	100		183,750	3,675,000
16	7085	52 M.	During failure (+ + + + +)	Rheumatic fever	Cardiac hypertrophy, mitral insufficiency, mitral stenosis chronic myocarditis, VPR*	Auricular fibrillation, C.H.F.	+	+	+	+	0	+	+	1			463,740	90	10	2,478,000	17,877,000
17	7323	27 M.	During failure (+ + + +)	Rheumatic fever	Cardiac hypertrophy, mitral stenosis and insufficiency, cardiac dilatation	Auricular fibrillation, C.H.F.	+	0	+	+	+	+	+	9				66		332,646,000	12,592,000
18	7222	36 M.	During failure (+ + + + +)	Syphilis	Cardiac hypertrophy, aortic insufficiency	Normal rhythm, C.H.F.	+	+	+	+	+	+	+	1	70	89	29,925		100	1,402,500	4,132,250

* C.H.F. = heart failure of the congestive type
VPR = right ventricular preponderance
APC = auricular premature contractions
VPC = ventricular premature contractions

in this table as well as in table 4.

TABLE 5

The urinary sediment of cardiac patients who have not suffered from heart failure of the congestive type

Case number	Hospital number	Age and sex	State with reference to heart failure	Diagnosis			Urea clearance	Phenolsulphonphthalein excretion in 2 hours	Sediment test (Addis)				
				Etiological	Anatomical	Physiological			Casts			Red blood cells, number in 12 hours	White blood cells, number in 12 hours
									Number in 12 hours	Hyaline	Granular		
24	7074	67 M.	Compensated	Arterio-sclerosis	Cardiac hypertrophy, mitral insufficiency, VPL	Normal rhythm, anginal syndrome	48	38	33,600	67	33	229,500	1,377,000
25	7067	71 M.	Compensated	Arterio-sclerosis	Cardiac hypertrophy	Normal rhythm	48	38	4,895	100		22,250	912,250
26	7180	65 M.	Compensated	Arterio-sclerosis	Cardiac hypertrophy, chronic myocarditis	Normal rhythm, anginal syndrome	15	50	0			123,750	247,500
27	H. II.*	54 M.	Compensated	Arterio-sclerosis	Slight cardiac hypertrophy	Normal rhythm, anginal syndrome	64	62	0			337,500	1,181,250
28	E. II.*	45 M.	Compensated 1 year later	Hypertension	Cardiac hypertrophy	Normal rhythm	137	36	6,100	100		119,000	1,788,000
				Hypertension	Cardiac hypertrophy	Normal rhythm	56	47	4,708	75	25	428,000	642,000
29	6323	52 M.	Compensated	Hypertension	Cardiac hypertrophy, VPL	Normal rhythm	82	61	80,000	8	92	24,000	96,000
30	7128	45 M.	Compensated	Hypertension	Cardiac hypertrophy	Normal rhythm	47	61	13,032		100	116,250	232,500

* Out-patient.

In such patients the number of casts was therefore increased, the average being 20 times that in normal subjects; the increase was not so great, however, as in cases of heart failure of the congestive type. The red cell and white cell counts were, however, definitely less; the average numbers though twice as great as in normal individuals were, nevertheless, within normal limits.

Urinary sediment and renal function. Renal function was studied in certain patients by means of the urea clearance test (3) and the excretion of phenolsulphonphthalein, but no relationship was observed between the degree of renal impairment and the number of casts, and red and white blood cells passed in 12 hours (tables 1, 2, 4 and 5).

DISCUSSION

In cardiac patients who exhibited *no signs of heart failure* Stewart and McIntosh (4) found that renal function measured by the Van Slyke index of urea excretion (5) and phenolsulphonphthalein excretion was usually normal, without reference to whether they had previously suffered attacks of congestive heart failure. Although normal in these respects, diminution of function was detected, nevertheless, in these individuals by means of the concentration and dilution tests (4); their kidneys could not in many instances excrete urine of high or of low specific gravity. This impairment was most frequent after attacks of heart failure of the congestive type. It is of course well known that decrease in renal function as measured by the urea index and phenolsulphonphthalein excretion is frequently observed during heart failure of the congestive variety and that return toward normal takes place as the signs of failure disappear (6). This is commonly attributed to congestion of the kidneys. No studies have yet been published of the number of formed elements in the urine of such patients. Increase in albumin in the urine and presence of casts, red blood cells and white blood cells in heart failure are, as abnormality in the case of chemical tests, commonly attributed to congestion. The actual numbers we have now counted. The cases studied are too few for statistical treatment, but the degrees of heart failure encountered were sufficient, we think, to suggest the limits within which the numbers may be expected to fall.

The most consistent finding was increase in the number of casts, the

average being 20 to 60 times greater than normal, depending on the severity of the disease. The number was smallest when failure of the congestive type had not occurred, somewhat greater when it had, though at the moment no signs were present, and greater still when they were. Granular casts which Addis (2) did not find in the urines of normal individuals were found in approximately half the cases.

During heart failure and after recovery increased numbers of red and white blood cells occur but almost as frequently they are within the normal range. When heart failure has not taken place red and white blood cells are within normal range, although the averages are approximately twice those of normal individuals. But in patients who have experienced heart failure the numbers are 10 to 15 times greater than normal. In Addis's (2) opinion it is only the appearance of a

TABLE 6

Comparison of average numbers of casts, red blood cells and white blood cells found in the urine in different states of heart disease

Casts,	No attacks of heart failure < Recovered from failure < During failure
Red blood cells,	No attacks of heart failure < Recovered from failure = During failure
White blood cells,	No attacks of heart failure < Recovered from failure < During failure

million or more red cells that can be regarded as significant. We could find no association, however, between the number of formed elements and the number of attacks of heart failure, nor between the degree of impairment of renal function (the urea clearance test and the phenol-sulphonphthalein excretion) and the number of formed elements in the urine. Etiology, so far as we could see, played no rôle, though the series is small to adopt an opinion on this point. Renal failure casts (Addis (7)) were not observed. On the whole it is surprising that the number of casts, red blood cells and white blood cells in the urines of cardiac patients is so small and that it is so little increased during cardiac decompensation (table 6), that there is, in short, so little alteration in function.

We wish to emphasize a point already made by Addis (2), namely

that the numbers of casts, red blood cells and white blood cells have no *individual significance*. In these cases furthermore the numbers of formed elements counted were small; they serve only to define the order of magnitude and to establish the limits or range of variation. It is only when they are large, as in Bright's disease, that the absolute numbers are significant. It is of course for this reason that the comparison between the stages of absence and presence of heart failure in the same patient sometimes shows decrease and sometimes increase; in this sense alone greater numbers of casts and white blood cells are found in the presence of heart failure, though the number of red blood cells is approximately the same.

SUMMARY

1. The number of *casts* found in 12 hours is usually increased in patients suffering from cardiac disease, although the number may be normal. If the average numbers are considered, the greatest numbers were passed by those patients suffering from heart failure of the congestive type; the numbers were fewer after recovery and fewer still in those who had never suffered from this illness. Granular casts were frequently found.
2. The number of *red blood cells* in the urine of patients who had experienced cardiac decompensation was frequently greater than the highest normal value, but within the limits in those who had never suffered from heart failure. The average number of red blood cells found in those cases which had never experienced heart failure was twice as great as that in normal individuals; in those who were suffering from heart failure or had recovered from it, however, the average number was 10 to 15 times as great as in normal individuals.
3. The number of *white blood cells* was normal in the urine of those patients who had not suffered from heart failure, but the average number was approximately twice the average observed in normal individuals. The number was usually within the normal range both during and after recovery from cardiac decompensation; the average number, however, was greater approximately 9 and 3 times respectively than that in normal individuals, the average being less in patients without heart failure than in those who had recently recovered from it and less than in those who were still suffering.

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THE VARIATIONS OF THE SPECIFIC GRAVITY OF THE PLASMA OF THE BLOOD AND THE MEANS AVAILABLE FOR ALTERING IT

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The protein content of the plasma of the blood can be calculated from the observed value of the specific gravity of the plasma with sufficient exactness for certain clinical purposes. This correlation was shown by Moore and Van Slyke (1), who found that the relationship is expressed by the formula: Grams total protein per 100 cc. plasma equal $343 (G - 1.0070)$, G being the observed specific gravity. They included measurements of the protein content of the plasma of normal individuals which were in agreement with those published by Linder, Lundsgaard and Van Slyke (2) in 1924, and by Salvesen (3) in 1926. These observers (2) (3) also published data showing that the plasma of individuals analyzed over a long period of time exhibits variations in the total protein content. That there are alterations in the direction of a decrease in the protein content of the plasma of the blood in pathological states has been shown by Linder, Lundsgaard and Van Slyke (2) and by Epstein (4) in nephritis, by Salvesen (5) in cirrhosis of the liver, in other diseases in which the liver is involved, in diabetes, in nephritis, and in anemia, and by others. Decreases both in specific gravity and in protein content of the plasma have been shown by Butterfield, Erdworm and Braddock (6) to be present in nephritis with edema and by Moore and Van Slyke (1) in nephritis with edema, that is to say, in Bright's disease.

The physiological variations in the proteins of the plasma and the means by which they may be altered experimentally have not been extensively studied. Greene and Rowntree (7) in 1924 demonstrated that the protein content of serum decreased after forced administra-

tion by mouth at half hour intervals of water to dogs in amounts equal to 5 per cent of the body weight. In experiments of 8 hours duration, amounts of water equal to 80 per cent of the initial weight of the animal were given. They also found that the amount of hemoglobin in the blood was reduced and that the decrease occurred most rapidly during the first 2 hours, but continued throughout the experiment; it was approximately 15 per cent. The total volume of circulating blood accordingly increased during the period of diminished hemoglobin concentration. Comparison of the refractive index and of the viscosity of the serum did not indicate alteration in the relative proportion of the serum proteins. Previous to this (1922), Lee, Carrier and Whipple (8) observed increase in the total plasma of the blood of dogs following ingestion of copious amounts of water.

In 1925, Cipriani and Moracchini (9) reported observations on the concentration of proteins in the serum of cardiac and of nephritic patients basing their measurements on the refractometric method. In 9 of 10 patients the protein content of the serum increased 7 to 15 per cent 4 hours after rising in the morning. Two normal individuals under similar conditions showed no change. No change was observed either in normal or in pathological cases during a control period of 4 hours in bed.

Recently, Thompson, Thompson and Dailey (10) published data which aid in interpreting the observations of Cipriani and Moracchini. They found that increase in protein content of the plasma of normal individuals took place while standing still, following a period of rest in the recumbent position. They observed also rise in the specific gravity of the plasma of the blood. It is unfortunately not possible to compare their data with those now being reported since unlike the technique employed by us oxalate was used as the anticoagulant, nor with those reported by Moore and Van Slyke (1) who used heparin.

Little information appears therefore to be at hand dealing with factors which influence the amount of protein in the plasma of the blood. Nor are there many studies on the methods which affect its specific gravity. For these reasons the present study was undertaken. Observations were made in the case of normal individuals, others in patients in hospital, and others, still, in dogs.

TECHNIQUE

The technique employed for measuring the specific gravity of plasma of the blood was that described by Moore and Van Slyke (1). Samples of blood were drawn from an arm vein, care being exercised to prevent stasis. Heparin was used as the anticoagulant. Evaporation was prevented before, during and after centrifuging by stoppering the tubes. A small bottle was used to weight the plasma. To obtain the specific gravity of the plasma the weight of the volume of plasma was divided by the weight of the same volume of water, both being at the same temperature. This procedure was performed in duplicate and checked within one or two in the fourth place of decimals. The method of calibration of the bottles for water was that described by Moore and Van Slyke (1).

OBSERVATIONS

The effect of anticoagulants on the specific gravity of the plasma of the blood. Because of the extensive use of potassium oxalate as an anticoagulant, it was used for this purpose on 3 occasions in order to compare its effect with that of heparin. Although oxalated plasma shows deviations from the values obtained in the case of heparinized plasma, there was in these few instances no constant difference between them. It is obvious therefore that the formula of Moore and Van Slyke is not applicable to oxalated plasma. If a larger series had been compared it would perhaps have been possible to correct the values obtained in the case of oxalated blood by inserting a constant factor in that formula, or else to derive a new formula for use with oxalated plasma.

Constancy of the specific gravity of the plasma of normal individuals during long and short periods. Linder, Lundsgaard and Van Slyke (2), Govaerts (11) and more recently Moore and Van Slyke (1) have shown that when the protein content of blood is low, as it is in certain cases of degenerative Bright's disease, it remains low over a long period of time and returns to a normal level slowly. Moore and Van Slyke (1) showed that the specific gravity of the plasma follows a parallel curve of recovery. We repeated these observations concerning the specific gravity of the plasma in 2 normal individuals on 3 successive days and on 4 successive days in a third. The blood was drawn at the same time each day with one exception when there was a delay of 15 minutes.

In this case although the food taken at breakfast differed, the specific gravity maintained a constant level. The greatest variation in any individual was 0.0003.

In patients suffering from cardiac disease, all in water equilibrium, blood was drawn at the same time each day in 5 cases. In each instance the level of the specific gravity remained constant. The greatest variation observed was 0.0004. The specific gravity of the plasma and by inference therefore its protein content remains remarkably constant.

We have found, as have others (Linder, Lundsgaard and Van Slyke (2), Salvesen (3)), that the specific gravity of the plasma of normal individuals changes appreciably over long periods of time. In one individual the plasma proteins¹ increased 0.5 gram per 100 cc. of plasma and in corresponding fashion the plasma specific gravity 0.0015 in a period of 14 months. In the case of a second normal subject, the specific gravity of the plasma increased 0.0011 during this time; whether a corresponding change in the total protein content occurred is not known.

On one subject the specific gravity was estimated 3 times during one day. In the middle of the day it was 0.0006 less than in the morning, or in the evening. It may therefore show slight fluctuations in a normal individual in the course of a day.

Comparison of the specific gravity and protein content of the plasma in arterial with that in venous blood. When the specific gravity of the plasma of arterial and of venous blood, taken at the same time, was estimated, as we had opportunity to do in 7 cases, that of venous blood was uniformly greater. The difference varied between 0.0006 and 0.0015 (table 1) and averaged 0.0010. In one instance in addition to specific gravity estimations the protein content, obtained by chemical analysis (Case 1), was found to be greater in venous blood, the difference corresponding to that observed in the specific gravity. The application of a tourniquet was not responsible for the difference, for in Case 4 (table 1) blood was drawn without the use of one. The number of red blood cells, the oxygen capacity of the blood (hemo-

¹ The proteins in the plasma were separated into albumin and globulin fractions by Howe's method (12) and determined by Van Slyke's gasometric micro-Kjeldahl procedure (13).

globin content), and the percentage of red blood cells to plasma were all slightly greater in venous than in arterial blood (Case 5); arterial blood appears in short to be more dilute than venous blood.

TABLE 1
Difference in specific gravity and protein content of the plasma of arterial and of venous blood drawn from the median cubital vein of the arm

Case number	Hospital number	Specific gravity of plasma	Total plasma protein	Albumin	Globulin	Hematocrit reading	Red blood cell count	Oxygen capacity of blood
			grams per 100 cc.	grams per 100 cc.	grams per 100 cc.	per cent cells	millions	volumes per cent
1	7106	Venous	1.0271	6.51	3.55	2.95		
		Arterial	1.0264	6.29	3.58	2.71		
		Difference	0.0007	0.22				
2	7054	Venous	1.0258					
		Arterial	1.0252					
		Difference	0.0006					
3	7182	Venous	1.0265					
		Arterial	1.0250					
		Difference	0.0015					
1	7106	Venous	1.0283					
		Arterial	1.0268					
		Difference	0.0015					
4	7255	Venous	1.0268					
		Arterial	1.0261					
		Difference	0.0007					
5	7318	Venous	1.0266			45.7		
		Arterial	1.0254			44.5		
		Difference	0.0012			1.2		
5	7318	Venous	1.0263			45.6	5.3	18.3
		Arterial	1.0256			44.2	4.6	17.7
		Difference	0.0007			1.4	0.7	0.6

The effect of the injection of salt solution intravenously upon the specific gravity of the plasma of the blood. The effect of diluting the blood by the intravenous infusion of physiologic sodium chloride solution is shown in experiments in dogs in which an amount of fluid was injected which varied between 40 and 80 per cent of the total blood volume

(table 2). In the first instance (dog 1), fifteen minutes after the infusion of 500 cc. having a specific gravity of 1.0052, the specific gravity of the plasma fell 0.0015 from 1.0238 to 1.0223. After 30 minutes a second infusion of 500 cc. was given. Fifteen minutes afterward the specific gravity of the plasma had fallen 0.0005 to 1.0218, a total of 0.0020 lower than before the experiment began. The following day, the specific gravity was still low, but had returned to the ante-infusion

TABLE 2
Effect on specific gravity of the plasma of the blood of injecting intravenously into dogs normal salt solution

Dog number	Date	Weight	Time	Specific gravity of plasma*	Remarks
1	January 7, 1930	13.25	1:55 p.m.	1.0238	500 cc. 0.9 per cent salt solution intravenously
			2:15 p.m.		
			2:30 p.m.	1.0223	500 cc. 0.9 per cent salt solution intravenously
			2:45 p.m.		
	January 8, 1930	13.75	3:00 p.m.	1.0218	
2	January 9, 1930		11:25 a.m.	1.0223	
	January 10, 1930		11:00 a.m.	1.0240	
	January 10, 1930		11:00 a.m.	1.0245	
	April 7, 1930	16.90	10:54 a.m.	1.0281	600 cc. 0.9 per cent salt solution intravenously†
			11:09		
			11:14	1.0243	

* Samples of blood were taken from a femoral artery since this was easier than drawing samples of venous blood.

† The specific gravity of this solution was 1.0055.

level on the next day, and two days later remarkable to relate was higher still.

In the case of the second dog (dog 2) the injection of 600 cc. of physiologic salt solution intravenously resulted in a decrease in the specific gravity of the plasma from 1.0281 to 1.0243. In this instance a shorter time (5 minutes) elapsed between injection and taking the second sample of blood and presumably less of the fluid which was injected had passed out of the blood stream. This experiment demon-

strates more accurately the degree of dilution of the blood which takes place after injection of salt solution than does the first.

Effect of hemorrhage on the specific gravity of the plasma. It has long been known that protein becomes deficient in the plasma of

TABLE 3
Effect of bleeding on the specific gravity of the plasma of the blood of dog 3

Date	Weight	Time	Specific gravity of plasma*	Total plasma protein	Albumin in plasma	Globulin in plasma	Albumin globulin ratio	Remarks
	kgm.			grams per 100 cc.	grams per 100 cc.	grams per 100 cc.		
January 7, 1930	9.75	11:00 a.m.	1.0231	5.34	2.94	2.40	1.2	No food since the day before. 400 cc. blood drawn from a femoral artery. Water allowed as desired†
		12:00 noon						
		4:15 p.m.	1.0194	4.41	2.29	2.12	1.0	Given food after this sample was taken
January 8, 1930	10.70	11:15 a.m.	1.0213					Sample taken before feeding
January 9, 1930		11:00 a.m.	1.0223					Sample taken before feeding
January 10, 1930		10:50 a.m.	1.0224					Sample taken before feeding
January 15, 1930		11:00 a.m.	1.0231					Sample taken before feeding

* Blood was drawn from a femoral artery since it was easier than taking samples of venous blood.

† This dog presumably had a total blood volume of approximately 1000 cc. Removing 400 cc. of blood therefore reduced the total blood volume to approximately 60 per cent of its initial value.

the blood on repeated bleeding (14). As a result of this procedure Leiter and McLean (15) showed that edema takes place in dogs although the washed corpuscles were returned to the blood stream. Later, when the proteins were again at normal concentration in the

after each voiding and the specific gravity of the plasma was estimated. In the first instance (Case 3) the specific gravity decreased 0.0002 from 1.0260 to 1.0258 (table 5) and in the second instance (Case 1), it fell 0.0003 from 1.0271 to 1.0269. In both instances the changes were small and may not be significant. Yet the lowest specific gravity of the plasma occurred at the time of the lowest specific gravity of the urine. The difference in the magnitude of the results between our observations and those in the animals of Greene and Rowntree is due,

TABLE 5
*Effect on specific gravity of the plasma of the blood of ingestion of 1000 cc. of water
(dilution test)*

Case number	Hospital number	Time	Amount of urine	Specific gravity of urine	Specific gravity of plasma	Decrease in specific gravity of plasma	Remarks
3	7182	7:00 a.m.	cc.	1.022	1.0260	0.0002	1000 cc. water
		7:05 a.m.					
		8:00 a.m.	55	1.017	1.0263		
		9:00 a.m.	485	1.002	1.0258		
		10:00 a.m.	175	1.005	1.0259		
		11:00 a.m.	88	1.011	1.0262		
1	7106	7:00 a.m.	420	1.020	1.0271	0.0003	1000 cc. water
		7:05 a.m.					
		8:00 a.m.	140	1.012	1.0271		
		9:00 a.m.	102	1.002	1.0268		
		10:00 a.m.	145	1.004	1.0271		
		11:00 a.m.	154	1.006	1.0273		
		12:00 noon	560	1.006	1.0269		

no doubt, to the fact that in their animals water was given to the point of intoxication. On the other hand we are unable to account for the difference in results between our observations and those reported by Strasser (20), for in certain instances he observed definite increase in the specific gravity of the plasma following the ingestion of 1000 cc. of water.

Effect of withholding fluid and giving a dry diet upon the specific gravity of the plasma of the blood. Observations were made in two patients during procedures which have as their object the secretion by the kidneys of urine of high specific gravity. The procedure as applied by

Stewart and McIntosh (19) is as follows: The patients are given 3 dry meals. Each meal consists of bread (toasted) 65 grams, butter 15 grams, eggs (scrambled) 100 grams, cream cheese 25 grams, and jam or jelly 15 to 20 grams. The caloric value of this meal is 600 calories, a total of 1800 calories. No water is given from midnight of the day preceding the procedure until its end. On the morning of the procedure the patient voids at 6 a.m. This specimen is discarded. He voids at 7 a.m., this specimen is saved. The dry meals are given at 7.30, 10.00 and 11.40 a.m. The patient voids at 9 and 11 a.m., and at

TABLE 6
Effect of withholding fluid and giving a dry diet on the specific gravity of the plasma of the blood

Case number	Hospital number	Time	Amount of urine	Specific gravity of urine	Specific gravity of plasma	Increase in specific gravity of plasma	Total plasma protein	Plasma albumin	Plasma globulin	A/G ratio
			cc.				grams per 100 cc.	grams per 100 cc.	grams per 100 cc.	
3	7182	7 a.m.	50	1.014	1.0259					
		9 a.m.	52	1.019	1.0269		6.56	4.21	2.35	1.7
		11 a.m.	80	1.020	1.0271					
		1 p.m.	77	1.024	1.0272	0.0013	6.73	4.07	2.66	1.5
		3 p.m.	84	1.025	1.0270					
1	7106	7 a.m.	90	1.018	1.0273					
		9 a.m.	48	1.020	1.0276					
		11 a.m.	75	1.023	1.0278					
		1 p.m.	156	1.020	1.0286	0.0013				
		3 p.m.	246	1.024	1.0283					

1 and 3 p.m.; each specimen is saved separately. The procedure ends after the specimen is collected at 3 p.m. The amount and specific gravity of each specimen are estimated. In normal individuals the specific gravity of the urine rises to 1.030.

In these patients samples of blood were taken immediately after each voiding and specific gravity of the plasma estimated. In the first instance (Case 3) the specific gravity rose 0.0013 from 1.0259 to 1.0272 (table 6). There was a corresponding rise of 0.17 gram per 100 cc. in the total protein content of the plasma from 6.56 grams per 100 cc. to 6.73 grams per 100 cc. In the case of the second patient

SPECIFIC GRAVITY OF PLASMA

TABLE 8

Effect on the specific gravity of the plasma of the blood of injecting intravenously gum acacia into a patient exhibiting edema due to nephrosis

Case number	Hospital number	Date	Specific gravity of plasma	Total plasma protein	Albumin in plasma	Globulin in plasma	Remarks
				grams per 100 cc.	grams per 100 cc.	grams per 100 cc.	
7	6644	April 19, 1929	1.0196	4.6	1.3	3.3	Before injection
			1.0199	3.6	1.0	2.6	After injection
		May 3, 1929	1.0187	3.8	1.2	2.6	Before injection
			1.0196	3.4	1.0	2.4	After injection

TABLE 9

Specific gravity and protein content of the plasma of cardiac patients

Case number	Hospital number	Date	Specific gravity of plasma		Total plasma protein	Albumin in plasma
					grams per 100 cc.	grams per 100 cc.
10	6784	February 9, 1929		1.0266	7.2	3.8
		March 12, 1929		1.0277	7.2	3.6
		May 27, 1929		1.0285		
11	6839	March 4, 1929		1.0268	6.1	4.0
		March 9, 1929		1.0294	7.0	4.0
12	6863	March 27, 1929		1.0243	6.0	4.0
13	6753	March 12, 1929		1.0287	7.3	3.5
14	6811	March 12, 1929		1.0284	6.9	4.0
		April 19, 1929		1.0291	7.6	4.6
15	6882	March 26, 1929		1.0285	6.8	3.3
		April 19, 1929		1.0294	7.5	3.3
16	6911	April 18, 1929		1.0268	6.2	3.4
		May 27, 1929		1.0268		
6	7182	January 9, 1930	7:00 a.m.	1.0259	6.6	4.2
			1:00 p.m.	1.0272	6.7	4.1
2	7106	January 20, 1930	Venous blood	1.0271	6.5	3.6
			Arterial blood	1.0264	6.3	3.6

jection was 1.0196 and the total protein 4.6 grams per cent (table 8). As soon as the infusion was finished the specific gravity of the plasma rose to 1.0199, but the total protein content of the plasma decreased to 3.6 grams per cent. Two weeks later the injection of 500 cc. of a similar solution resulted in similar changes. The increase in specific gravity was due presumably to the presence of gum acacia; the fall in the concentration of protein is explained partly by dilution of the plasma resulting from the volume of solution injected and partly by the entrance of fluid into the blood stream due to presence there of the hypertonic glucose solution (18).

Correlation in cardiac patients of the protein content and of the specific gravity of the plasma of the blood. Moore and Van Slyke (1) demonstrated a close agreement between the specific gravity of the plasma and protein content in normal individuals and in patients suffering from Bright's disease (hemorrhagic, degenerative and arteriosclerotic) whether edema was or was not present. In a corresponding fashion we have measured the protein content and the specific gravity of the plasma of a number of cardiac patients both those exhibiting edema as well as those free of it. The specific gravity of the plasma and the plasma protein content were both within the normal range (table 9). A correlation as in nephritic patients was found between the specific gravity and the protein content. If these data are inserted in figure 3 of Moore and Van Slyke's paper (1) now reproduced as figure 1, the points lie about the straight line drawn by them. In calculating the plasma protein content from the observed specific gravity their formula may therefore be used, in which plasma protein expressed in grams per 100 cc. of plasma equals $343 (G - 1.0070)$, where G is the observed specific gravity.

DISCUSSION

We have presented data which show striking constancy in the specific gravity of the plasma of the blood in normal individuals as well as in the plasma proteins. We have also presented the results of our attempts to alter the specific gravity of the plasma.

That the specific gravity of the plasma and correspondingly of the concentration of proteins in the plasma vary within such small limits (0.0003) in normal individuals and in cardiac patients (0.0004) is sur-

prising. Other observers (Linder, Lundsgaard and Van Slyke (2), Salvesen (3)) have called attention to variations which occur in the protein content of the plasma over long periods of time. We have confirmed these observations and have found in addition that changes in the specific gravity parallel changes in the concentration of plasma protein. During the course of a day the specific gravity was found to vary 0.0007 in one individual, being lowest in the middle of the day. How much variation, and presumably it is very little, is due to changes in water balance and how much to concentration of the blood due to standing and walking is not known. Thompson, Thompson and Dailey (10) believed that increase in the concentration of protein and increase in specific gravity of the plasma occur when the position of the body is changed from recumbent to standing, due to loss of fluid (approximately protein free) from the blood amounting on the average to 11 per cent of the total plasma volume. Their studies were based on estimations of blood volume. Cipriani and Moracchini (9) likewise observed increase in serum protein concentration in cardiac and nephritic patients 4 hours after rising, but observed no change in normal individuals. They employed the refractometric method. It may be recalled that Linder, Lundsgaard and Van Slyke (2) compared the results obtained by the refractometric method for the estimation of protein with those gained from chemical analysis of the blood by the Kjeldahl method and found that they differed as much as 1.5 gram of protein per 100 cc. of plasma in normal individuals.

The procedures which we have used to alter the concentration of protein and the specific gravity of the plasma have had as their basis dilution or concentration of the circulating blood by changing its fluid content; they were not dependent upon alteration of the total protein content of the circulating plasma. The decrease in specific gravity of the plasma following the intravenous injection of hypertonic solutions of glucose takes place with surprising rapidity, the maximum reduction being present a few minutes after the infusion is finished. The dilution of the plasma which is indicated by the decrease in specific gravity corresponds approximately to the increase in plasma volume of the circulating blood which Smith demonstrated in dogs under conditions similar to ours. The return of the specific gravity to its original level occurs with the removal of the excess fluid from the blood.

It is a fact that the organism quickly restores to a normal level a lowered concentration of protein in the plasma which has been artificially induced. This is the situation following large hemorrhages. If, on the other hand, deficiency of protein in the plasma of the blood is a manifestation of disease, as is the case in nephritis with edema, the low level is maintained over long periods of time (1); we are at present without measures of increasing the protein content of the plasma within a short time. The attempts to increase the specific gravity of plasma independently of its protein content by intravenous injections of gum acacia were only partially successful, since the addition of relatively large amounts of it to the circulating blood resulted in only slight rises in the specific gravity.

SUMMARY

1. The anticoagulant used has an effect on the specific gravity of the plasma. If data are to be compared with those published by Moore and Van Slyke, heparin must in consequence be used.

2. It has been demonstrated that the specific gravity of the plasma and, on the basis of Moore and Van Slyke's observations, the plasma proteins of normal individuals show remarkable constancy for short periods (days) provided the samples of blood are taken at the same time each day.

3. In patients suffering from heart disease who were in water balance the maximum and minimum daily variations in the specific gravity were 0.0004 and 0 respectively.

4. In two normal individuals the variations were 0.0015 and 0.0011 over a period of 14 months. There was corresponding variation in the protein content of the plasma.

5. The specific gravity of the plasma of a normal individual varied 0.0007 in the course of a day.

6. The protein content and the specific gravity of the plasma of arterial blood is less than of venous blood.

7. The injection intravenously in dogs of normal salt solution resulted in decrease in the specific gravity of the plasma of the blood. The administration of water 1000 cc. by mouth to human beings decreased the specific gravity of the plasma, however, only slightly.

8. During "concentration tests" two patients exhibited increases (0.0013) in the specific gravity of the plasma of the blood.

9. Hemorrhage was followed by decrease in specific gravity of the plasma, and a corresponding decrease in the plasma protein concentration. Return to the initial level occurred 4 days after bleeding.

10. The intravenous injection of hypertonic solutions of glucose resulted in decrease in the specific gravity of the plasma.

11. The injection of gum acacia in glucose solution increased the specific gravity of the plasma slightly, but the volume of fluid injected and the dilution resulting from the presence of hypertonic glucose solution decreased the protein concentration.

12. The specific gravity and the protein content of the plasma of patients suffering from cardiac disease are within the range found in normal individuals.

13. In those cardiac patients exhibiting edema as well as in those in whom this sign is not present, there is a linear relationship between the specific gravity and the total protein content of the plasma which is expressed by the formula of Moore and Van Slyke.

CONCLUSIONS

1. The specific gravity of the plasma and parallel with it the plasma proteins show only small fluctuations in normal individuals.

2. Bleeding, intravenous injection of normal salt solution, the intravenous injection of hypertonic glucose solution, and to a much less extent, the ingestion of water may bring about dilution of the plasma and in consequence alterations in the normal specific gravity and in the concentration of the plasma proteins; on the other hand, withholding fluid and ingestion of a dry diet may raise the specific gravity and the concentration of the proteins. The ingestion of fat also decreases the specific gravity of the plasma.

3. The specific gravity and protein content of the plasma of patients suffering from cardiac disease irrespective of the presence of edema was greater than that associated with edema in nephritic patients.

4. The protein content of the plasma of cardiac patients may be calculated from the observed value of the specific gravity of the plasma by applying the formula published by Moore and Van Slyke (1) for use in normal individuals and patients suffering from Bright's disease.

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THE OPTICAL ACTIVITY OF CEREBROSPINAL FLUID IN SUPPURATIVE MENINGITIS, AND ITS LACTIC ACID, SUGAR, AND CHLORIDE CONTENT

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This study deals with the concentrations of lactate, chloride, and reducing substances, and with the optical activity of ultrafiltrates from 49 specimens of cerebrospinal fluid from 9 patients with meningitis. In 6 instances comparisons of the concentrations of lactate, chloride, and reducing substance in blood and spinal fluid are given. The change in concentration of lactate, and reducing substances, and in the optical activity in four specimens of cerebrospinal fluid during incubation at 37° for 1 or 2 days is reported.

In an earlier study (1) we showed that the greatest part of the levorotatory substances detected in protein free ultrafiltrates from blood, consists of the salts of d-lactic acid present in the blood or formed in vitro as the result of the glycolysis of the sugar. In this study, when sufficient fluid was available, polarimetric observations were made to determine whether changes in the rotation observed are correlated with the lactates found in the cerebrospinal fluid in meningitis.

LITERATURE

Normal cerebrospinal fluid

An examination of the literature has revealed analyses for sugar or lactate in cerebrospinal fluid from individuals supposedly quite normal in 28 subjects by 5 observers. The results are shown in table 1. In addition to these there can be gathered from the literature data from various authors on patients in whom it was reasonably assumed by the authors that the disease from which the patient suffered would not be associated with abnormal values for the constituents of the cerebrospinal fluid under consideration.

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Reducing substance. The amounts of reducing substance (calculated as dextrose) found in cerebrospinal fluids supposedly normal in this respect, vary from 27 to 100 mgm. per 100 cc. By far the greater number, however, are within the range from 45 to 80 mgm. and the average value lies between 60 and 70 mgm. per 100 cc. (4-12, 22-25). The reducing substance of cerebrospinal fluid has been found lower than that of blood. The ratio has been reported as from 0.40 to 0.81 where fluid and blood have been obtained at approximately the same time (11-13, 24). It has been reported that the reducing substance of cerebrospinal fluid varies with that of blood (11-16, 25), rising after meals (12-13), falling after insulin (26), and maintaining a high level in diabetes and other conditions attended with hyperglycemia (14, 23). On the other hand the range of the ratio given above indicates that the correlation is subject to considerable variation. Whereas 10 to 30 mgm. per 100 cc. of the reducing substance of blood appears to be not glucose

TABLE 1
Lactate and reducing substance in normal cerebrospinal fluids

<i>Lactate and reducing substance in normal cerebrospinal fluids</i>						
Reducing substance			Lactate			Observer and reference
Number of specimens	Range	Mean	Number of specimens	Range	Mean	
	<i>mgm. per 100 cc.</i>	<i>mgm. per 100 cc.</i>		<i>mgm. per 100 cc.</i>	<i>mgm. per 100 cc.</i>	
3	64-83	72				Coope (23)
6	53-74	61				Thalhimer and Updegraff (2)
6	35-78	63	6	6-11	9	Osnato and Killian (8)
8	54-82	70	8	6-11	8	Nishida (3)
5	48-59	54	5	13-17	15	Geldrich (21)

(29-32), the non-glucose reducing substance of the cerebrospinal fluid is much less in amount (27, 28). However, most of the non-glucose reducing substance of the blood is contained in the cells (30-33) and if, as has been claimed (31, 32, 34), the glucose of the blood is in higher concentration in the plasma than in the cells, the ratio of glucose in cerebrospinal fluid to glucose in plasma is probably smaller than the figures given above.

Lactate. Nishimura (36) found amounts from 11.7 to 18.0 mgm. per 100 cc. in the apparently normal fluids from cases of encephalitis and cerebrospinal syphilis. Glaser (37) analysing thirteen rather carefully selected fluids found values from 11 to 27 mgm. and averaging 19 mgm. per cent. Several others besides those mentioned above and in table 1 have determined the lactate content of normal or nearly normal fluids (15, 20). The changes of blood lactate due to muscular exercise and other causes have recently been reviewed by Jervell (38). Wittgenstein and Gaedertz (39) showed that the cerebrospinal fluid responds to increases in blood lactate resulting from exercise, though the response in the fluid is delayed in a man-

ner similar to that observed by Halliday (12) and Goodwin and Shelley (13) in the case of changes of sugar content. The changes of blood and fluid lactate content in eclampsia appear to resemble those following exercise (40).

In view of the above, and the paucity of data on resting subjects, lactic acid concentrations from 6 to 30 mgm. per cent must be considered within the normal range, though the upper part of the range is probably too high for subjects at rest. Chevassut (41) denies the presence of lactates in cerebrospinal fluid in vivo, but the weight of evidence seems against her interpretation.

Chloride. The normal average chloride content of cerebrospinal fluid as determined by different observers is remarkably consistent. Hamilton (42) found 124 m.Eq. per liter, Becker (5) 125, while Stewart (24) and Neale and Esselmont (10) found 124 and 125 respectively in children over five years of age. The great majority of the analyses on normal or supposedly normal fluids lie between 121 and 130 m.Eq. per liter.

The history of investigation into the origin of cerebrospinal fluid has recently been outlined by Levinson (35). Fremont-Smith (43) has summarized the evidence favoring the view that the fluid is a dialysate or filtrate.

Cerebrospinal fluid in meningitis

Reducing substance. It has long been known that in certain diseases the amount of reducing substance in the cerebrospinal fluid is greatly diminished. It is almost invariably low in purulent meningitis (2, 3, 7-11, 13, 17-23, 44) and occasionally in syphilis of the central nervous system, especially in untreated cases (5, 13). Neale and Esselmont (10) following the changes in concentration of reducing substance during the course of several types of meningitis reported characteristic changes in the various types. Geldrich (21) noted that in tuberculous meningitis there is occasionally a rise in the concentration from a low value to normal or nearly normal figures shortly before death.

Lactate. Comparatively few studies have been made on the lactate content of cerebrospinal fluid in meningitis. Nishimura (36) reported a slight increase in tuberculous meningitis, and Killian (19) and Osnato and Killian (8) noted high lactic acid concentrations (up to 84 mgm. per 100 cc.) in cases of several different types of meningitis. They found that in subjects responding to treatment the values became normal previous to discharge, while in those terminating in death the lactic acid content remained high. Glaser (37) reported 91 mgm. per 100 cc. in a case of influenzal and 150 mgm. per 100 cc. in a case of streptococcic meningitis, and Scheller (20) found an elevation of lactic acid in five cases of tuberculous and one of pneumococcic meningitis. Nishida (3) reported elevation of the lactic acid content of cerebrospinal fluid in cases of meningococcic, tuberculous and pneumococcic meningitis. More recently, Geldrich (21) published a series of cases of tuberculous meningitis in which the lactic acid was consistently elevated. Fasold and Schmidt (45) and Margreth (46) also have reported an elevation of lactic acid in cases of meningitis.

All investigators agree that the lactic acid content of cerebrospinal fluid is increased in suppurative and tuberculous meningitis, the amount of increase reported varying with the severity of the disease. Geldrich (21) and Garcia, Killian, and De Sanctis (47) agree that the lactic acid content of the fluid is of value as a guide to the patients progress, and of more value in this respect than the sugar content of the fluid. Geldrich observed that during a large part of the illness, the sum of glucose and lactic acid tends to remain within or close to normal limits, but as a fatal termination approaches, the sum usually increases. He found a rather steady but slow increase in lactic acid of the cerebrospinal fluid during the progress of tuberculous meningitis, until during the last week it would rise more rapidly, often to 120 mgm. per 100 cc.

Chloride. A lowered chloride concentration in cerebrospinal fluid in tuberculous meningitis seems generally recognized. Nowicka (48) found in sixty cases of tuberculous meningitis, values ranging from 87 to 116 m.Eq. per liter with a mean of 102, in general falling in the final stages nearly to the lower figure. In meningococcic meningitis the range was 108 to 120 m.Eq. per liter. Wilcox, Lytle and Hearn (11) find a mean concentration of about 109 for tuberculous meningitis, and report the blood chlorides also lowered though not to so great an extent. Linder and Carmichael (49), however, find that the ratio of cerebrospinal fluid chloride to blood chloride is unaltered in meningitis and conclude that the fall of chloride concentration in the cerebrospinal fluid in meningitis is dependent on a similar fall in the chloride concentration of the serum. Neale and Esselmont (10) find that the changes in the chloride concentration of the fluid during the progress of the disease show special features in each type of meningitis. The range of values they report are, however, similar to those referred to above.

METHODS

The specimens of spinal fluid, except where otherwise noted, were obtained by lumbar puncture and were taken immediately to the laboratory. When the puncture was made at night, the fluid was kept in the refrigerator until the following morning, but ordinarily not over an hour elapsed between collection and analysis. For polarimetric readings it was necessary to remove proteins. Ultrafiltration through collodion sacs was the method used, and for the sake of uniformity this procedure was carried out preliminary to all analyses. The filtration was rapid and only occasionally required over twenty or thirty minutes.

Reducing substances ("sugar") were determined by the method of Folin and Wu after appropriate dilution, and chlorides by that of Van Slyke (50). The lactic acid determinations were made by the method of Friedemann, Cotonio and Schaffer (51), and were carefully controlled by determinations made on blanks and on known quantities of pure zinc lactate. The green light of a mercury vapor lamp was used for polarimetric readings. The optical activity is expressed in terms of the milligrams of dextrose per 100 cc. required to give the same rotation, using 63.03° as the specific rotation of dextrose for a wave length of 5416\AA . Details

regarding the instrument and the method of preparing and using the collodion sacs are given in the earlier papers (1, 52, 53). By expressing the optical activity in terms of dextrose either plus or minus, comparison between the optical activities of fluids of differing actual dextrose content (as determined by reduction) can be made more conveniently than when rotation is expressed in degrees.

The fluids were tested with phenol red before the polarimetric observations to be certain that they were not acid in reaction. This was necessary in view of the change which marked variations in the pH exert upon the optical activity of lactates (1).

RESULTS

Normals

A few determinations were made on cerebrospinal fluids collected in the Out-Patient Department for Wassermann determinations. While obviously none were from persons in perfect health, fifteen of them were apparently normal fluids from persons suffering from a variety of diseases but with presumably normal chemical composition of the cerebrospinal fluid. Among them are patients admitted for diagnostic study as well as patients suffering from heart disease, paralysis agitans, chronic maxillary sinusitis, and cerebrospinal syphilis.

In these fifteen cases the sugar concentration in the fluid varied from 43 to 95 and averaged 66 mgm. per 100 cc., and the lactic acid varied from 12 to 31 and averaged 19 mgm. per 100 cc.

Meningitis

Our data include one case of pneumococcic, one of tuberculous, three of streptococcic and four of meningococcic meningitis. A total of 49 specimens of cerebrospinal fluid from these nine cases were examined. Except for one case of streptococcic and three of meningococcic infection, data are not available before the day previous to death. Forty of the fluids examined were from these four cases.

In neither of the cases showing eventual recovery did we secure suitable specimens for analysis during the period of improvement and return to normal. In Case 6, no punctures were made later than those here recorded. In Case 1 a few were made but the fluids were not analyzed as treatment at this period was by irrigation of the entire canal with saline.

It was contended by Goodwin and Shelley (13) that a low sugar

concentration in the cerebrospinal fluid after intraspinal administration of antimeningococcus serum is of no diagnostic value. Analyses of serum prepared by two manufacturers, showed reducing values of 12 and 12 mgm. per 100 cc., lactic acid concentrations of 70 and 50 mgm. per 100 cc. and chloride concentrations of 95 and 100 m.Eq. per liter respectively. This is a deviation from the normal values for spinal fluid in the direction reported in cases of meningitis. A comment as to the serum treatment is therefore in order. In Cases 1, 2, and 6 from 12 to 30 cc. of antimeningococcus serum were injected usually twice daily immediately after removal of the specimen for analysis. Thus an average period of 12 hours elapsed between serum treatment and removal of the specimen. In Case 7, 15 cc. of sera were injected on each occasion, but at longer intervals. The specimens recorded were collected from one to nine days after the last previous treatment. In Case 9, one injection of 25 cc. of antistreptococcic serum was made, five hours previous to the collection of the last antemortem specimen.

Reducing

The sugar content in our series of cases is generally lowered, half the specimens showing a sugar content of 20 mgm. per 100 cc. or less. The only marked exception is in Case 5 where the sugar in the fluid was 90 mgm. per 100 cc. In this patient the blood sugar was 254 mgm. per 100 cc. and a relatively high fluid sugar was therefore to be expected.

Lactic acid

✓ The lactic acid concentration increased in some cases eight to ten fold, four times becoming greater than 150 mgm. per 100 cc. in the last specimen prior to death.

Only seven observations showed less than 30 mgm. of lactic acid per 100 cc. Of these, six are during the favorable period of Case 1.

We do not find the constancy in the sum of lactic acid and sugar concentrations reported by Geldrich. In Case 1 for example the sum fell below the normal range and in Case 6 the sum exceeded 100 on two successive days but the patient eventually recovered. The sum does, it is true, rise to a high figure previous to death.

Chloride

Thirty chloride determinations were made and the results range from 77 to 126 m.Eq. per liter being under 121 in 83 per cent of the observations and under 110 in 50 per cent. We therefore find, in agreement with others, that the chloride concentration is lowered, sometimes very considerably, in suppurative meningitis. Only five observations fell within the range 121-130 m.Eq. per liter and three of these occurred in a patient (Case 6) who finally recovered.

TABLE 2
Comparison of concentrations in blood and cerebrospinal fluid*

Case	Day	Lactic acid			Reducing			Chloride		
		Blood	Fluid	Ratio $\frac{F}{B}$	Blood	Fluid	Ratio $\frac{F}{B}$	Serum	Fluid	Ratio $\frac{F}{S}$
		mgm. per 100 cc.	mgm. per 100 cc.		mgm. dextrose per 100 cc.	mgm. dextrose per 100 cc.		m. Eq. per liter	m. Eq. per liter	
2	2	40	115	2.9	74	15	0.20			
5	1				254	90	0.39			
6	9	29	69	2.4	100	22	0.22	94	113	1.20
	10	25	71	2.8	91	18	0.20	94	116	1.23
8	2	35	156	4.5	179	10	0.06	92	109	1.19
	3	23	171	7.4	152	20	0.13			
Average.		30	116	4.0	142	29	0.20	93	113	1.21
Normal.		23	19	0.8	100	66	0.66	103	124	1.20

* Lactic acid and reducing on whole blood; chloride on serum.

Ratio of concentrations in blood and cerebrospinal fluid

On six occasions we examined blood and cerebrospinal fluid taken almost simultaneously from cases of meningitis. The results of these determinations are shown in table 2, together with the approximate mean normal values. In the case of lactic acid, the ratios vary from 2.4 to 7.4 contrasted with a normal value of 0.8. The normal ratio for sugar is about 0.66 but in our cases of suppurative meningitis the ratios were from 0.06 to 0.39. In the case of chloride, the ratio found by us is normal, a confirmation of the observations of Linder and Carmichael mentioned earlier (49).

Optical activity

Few observations of the optical activity of cerebrospinal fluid have been recorded and none to our knowledge, have previously been made on fluids from patients with meningitis. Lundsgaard and Høllboll (54) reported six fluids from patients with normal carbohydrate metabolism. The glucose content varied from 68 to 83 mgm. per 100 cc. as determined by reduction and from 18 to 37 mgm. per 100 cc. by optical activity. On allowing the fluids to stand for 48 hours, the reducing values remained nearly constant, while the polarimetric values increased to approximate agreement with them. Hagedorn (55) in similar experiments in a larger series of fluids found reduction values corresponding to from 43 to 70 mgm. of glucose per 100 cc. The optical activities were, however, nearly zero, and standing for 48 or 72 hours did not alter the findings. The increase of optical activity of cerebrospinal fluid on standing, described by Lundsgaard and Høllboll could not be confirmed.

In view of the above disagreement of results, Gram, Nielsen and Rud (56) made a similar study. In three out of eleven specimens an increase of optical activity greater than the experimental error, was observed on standing. They suspected that the small amount of protein present in cerebrospinal fluid might be altered or precipitated on standing and cause an increase in rotatory power. Their results in four fluids of patients with normal carbohydrate metabolism from which the proteins were precipitated by means of lead acetate together with a series in which the glucose was removed by dialysis, readings made, and the protein then precipitated, indicated to them that the wide discrepancy between the polarimetric and reducing methods of determining glucose was caused by the presence of proteins, and that alteration or disappearance of the proteins was the cause of the rise in optical activity reported by Lundsgaard and Høllboll. Their data showing the results of polarimetric observations after the precipitation of proteins, are the only ones comparable to our results after ultrafiltration. They report glucose contents, based upon the optical activity, of 50, 58, and 61 mgm. per 100 cc., whereas those estimated by reduction were 65, 76, and 64 respectively.

In our cases of suppurative meningitis, the cerebrospinal fluid was found to be actually levorotatory in 13 of 19 instances. This fact,

together with the high lactate content of the fluids and the absence of proteins, suggests that the lactates are present in the form of salts of d-lactic acid, all of which are levorotatory. We have found this to be the case in ultrafiltrates of glycolysed blood. In order to show the

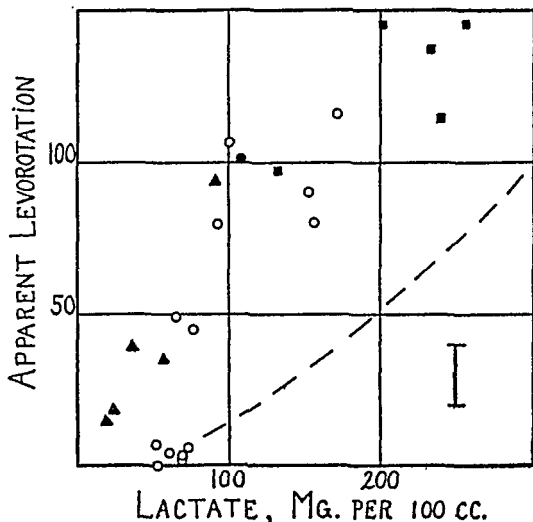


FIG. 1. THE APPARENT AMOUNT OF LEVOROTATION (EXPRESSED IN MILLIGRAMS OF DEXTROSE PER 100 CC.) IN SPINAL FLUID COMPARED WITH ITS LACTATE CONTENT

Circles represent fluids from lumbar puncture; shaded circles, from cisternal puncture; squares, postmortem, and triangles, after incubation. The approximate line for blood is indicated by dashes, and the error of the polarimetric reading (± 10 mgm.) by the vertical line.

relation between the lactate concentration and the apparent amount of levorotation, the data bearing upon it are plotted in figure 1. The apparent amount of levorotation is a calculated value, being the milligrams of dextrose it would be necessary to add to 100 cc. of the fluid to make the optical activity of the fluid equal that of a pure solution of

dextrose of the concentration indicated by the reducing determinations. It will be observed that the points tend to fall along a curve similar to, but not identical with that drawn for blood in our previous paper (1). The six points lying together somewhat apart from the others are those (five of them from Case 6) in which the total optical activity was dextro-rotatory. In the curve drawn from blood we obtained, by glycolysis, a figure for the non-glucose reducing substances and subtracted this from the reducing value in computing the apparent levorotation. In calculating the apparent levorotation of the spinal fluid as shown in figure 1, no allowance is made for non-glucose reducing substances. That they are absent is probably not true but they can hardly introduce an error that invalidates our conclusion drawn from figure 1 that there is a relationship between the apparent levorotation and the lactate concentration.

Glycolysis

Specimens of normal cerebrospinal fluid handled in a manner to avoid contamination, showed no alteration in reducing substance on standing at room temperature or in the incubator as long as 48 hours. This is in agreement with the recent report of Nielsen (57) who found that the loss of sugar frequently did not begin until a much longer time had elapsed than we allowed. Chevassut (41) found glycolysis occurring promptly and rapidly in normal fluid. Fasold and Schmidt (45) found insignificant changes of sugar and lactate in fluids from cases of meningitis.

Four specimens of fluid from a case of meningococcic meningitis were allowed to stand for 24 or 48 hours in an incubator. The concentration of reducing substance was small at the start and remained virtually unchanged. The lactate content, however, originally large, became less, and the optical activity showed a correlated diminished amount of levorotation. The data are shown in table 3 and are included in figure 1 as triangles. The fluids were allowed to stand as drawn, but the protein and cells were removed by ultrafiltration before the polarimetric reading.

This change in optical activity is in the same direction as reported by Lundsgaard and Hølboll and in three instances by Gram, Nielson and Rud. No lactic acid determinations were made in those studies.

While the presence of proteins would be a disturbing factor in any study of optical activity, our experiments indicate that alterations in lactic acid content may be of considerable significance.

TABLE 3
The effect of incubation in cerebrospinal fluid in meningitis. Case 7

Day of disease	Incubated	Lactic acid	Reducing value (dextrose equivalent)	Rotation (dextrose equivalent)	Apparent levo- rotation (dex- trose equivalent)
	<i>days</i>	<i>mgm. per 100 cc.</i>	<i>mgm. per 100 cc.</i>	<i>mgm. per 100 cc.</i>	<i>mgm. per 100 cc.</i>
15	Fresh	65	15	-34	49
	1	36	10	-29	39
21	Fresh	108	24	-77	101
	1	93	20	-73	93
	2	19	15	0	15
27	Fresh	93	18	-61	79
	1	23	18	0	18
30	Fresh	172	15	-101	116
	1	56	15	-20	35

DISCUSSION

Insufficient evidence is available to satisfactorily establish the mechanism of formation of normal cerebrospinal fluid and the relation between the concentration of its chemical constituents and the same constituents of the plasma, although evidence for the importance of dialysis or filtration through the membranes of the choroid plexus is impressive. The explanation of pathological observations is obviously even more complex. The apparently greater disturbance of glucose and lactic acid equilibrium than of chloride equilibrium between plasma and fluid may be due to the conversion of glucose to lactic acid by leucocytes and bacteria, probably especially the former.

CONCLUSIONS

1. Lactic acid determinations on the cerebrospinal fluid give striking evidence of abnormality in cases of suppurative meningitis and the lactic acid concentrations are more or less related to the severity of the clinical symptoms.

dextrose of the concentration indicated by the reducing determinations. It will be observed that the points tend to fall along a curve similar to, but not identical with that drawn for blood in our previous paper (1). The six points lying together somewhat apart from the others are those (five of them from Case 6) in which the total optical activity was dextro-rotatory. In the curve drawn from blood we obtained, by glycolysis, a figure for the non-glucose reducing substances and subtracted this from the reducing value in computing the apparent levorotation. In calculating the apparent levorotation of the spinal fluid as shown in figure 1, no allowance is made for non-glucose reducing substances. That they are absent is probably not true but they can hardly introduce an error that invalidates our conclusion drawn from figure 1 that there is a relationship between the apparent levorotation and the lactate concentration.

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been troubled with chills and drowsiness for the past three weeks. A discharge from the nose, chronic for ten years, ceased January 8. Spinal fluid was clear, colorless, with cells 119 per cubic millimeter; 80 per cent lymphocytes, 20 per cent red blood cells, no polymorphonuclears. January 17, cells of fluid were 687. Became steadily worse and died January 18. Autopsy showed tubercles in lungs, brain, and along walls of the meningeal vessels.

Case 4. Unit history number 8715. Male, age 18. Admitted December 28, 1928 with a severe acute mastoiditis complicated by an acute nephritis and a gonococcal urethritis. Symptoms of meningitis developed January 20 and patient died January 22, 1929. Almost pure growth of hemolytic streptococci was obtained from spinal fluid and from swab of left ear. Blood leucocytes were 14,000 to 16,900 but blood culture was negative.

Case 5. Unit history number 9076. Colored laborer, age 29. Illness began on December 28, 1928 with pain in left eye and left side of head. January 2, 1929 was treated conservatively in Out-Patient Department for frontal sinusitis. January 23 convulsions began, beginning in right arm. Brought to hospital unconscious. Convulsions became generalized. Right arm and leg were paralyzed, with diminution or absence of reflexes. Lumbar punctures: pressure 4 mm. Hg, cloudy, 4,200 cells, 95 per cent polymorphonuclears. Blood sugar 254 mgm. per 100 cc., urea nitrogen 13.4. Temperature ranged from 37.4 to 40.4°C. Death occurred on January 24th, called the second day of meningeal involvement. Diagnosis at autopsy: left frontal abscess, which had extended into the ventricle to produce ependymitis and meningitis. Organism was reported as an hemolytic streptococcus.

Case 6. Unit history number 9088. White girl, age 7. Admitted to hospital January 23, 1929, called the second day of disease with classical picture of meningitis. Marked rigidity of neck, Kernig's sign and photophobia were present. Antimeningococcic serum was given intravenously and intraspinally. The fifth day, her condition was much better. The ninth day she was not improving, cough troublesome. On the twelfth day, a cisternal tap was made and from that date improvement was continuous. Discharged March 25. Temperature 37.8 to 40.4°C. during first eight days, normal later. Meningococci recognized in spinal fluid and cultured. Our data cover the decline and the beginnings of improvement.

Case 7. Unit history number 9204. White boy, age 8. Admitted January 30, 1929. Diagnosed acute appendicitis. Appendectomy was performed. Appendix found slightly enlarged. Bloody serous fluid was in peritoneal cavity. January 31, called the first day of disease, cheeks were flushed, patient apathetic, and photophobia and definite cervical rigidity were present. Heart and lungs were normal. Questionable Kernig's on left was demonstrated. Positive Babinski on

2. In suppurative meningitis the ultrafiltrate of the cerebrospinal fluid is usually levorotatory. The parallelism between degree of levorotation and lactic acid concentration suggests that the latter is present in the form of levorotatory d-lactates, which is the same form that has been found in glycolysed blood.

3. The ratio of the concentration of chloride in the blood to that in the cerebrospinal fluid in suppurative meningitis was not found altered from the normal.

4. The sugar and lactic acid ratios were markedly altered, the cerebrospinal fluid being relatively high in lactic acid content and low in sugar content

PROTOCOLS

Case 1. Unit history number 8658. White male, age 20. Admitted December 25, 1928, called the 2nd day of disease, complaining of headache, fever and occasional chills. Lumbar puncture revealed a cloudy fluid, pressure 22 mm. Hg, cells 12,500 per cubic millimeter largely polymorphonuclear, few organisms. Antimeningococcic polyvalent serum was introduced. The following day a positive Kernig's sign and distinct rigidity of the neck were noted. Under serum treatment the fluid cell count fell to as low as 300; his temperature became normal on the fifth day of disease and improvement was noted for several days thereafter. His general condition then steadily grew worse until the 21st day of disease when improvement was again noted. Vision was clear, no headache; he looked and talked better. That afternoon the fluid from the lumbar puncture ran slowly and showed 14,400 cells. On the 22nd day no fluid could be obtained; apparently there was a block. On the 23rd day, cisternal and lumbar punctures were made and the canal irrigated with saline. This was repeated the following day, and from that point recovery was rapid and continuous. Patient discharged March 12, 1929. Temperature during stay in hospital was 37.5 to 39.8°C. Spinal fluid culture showed meningococci. Our data include the early improvement and later decline, but do not include any figures during the final improvement and recovery.

Case 2. Unit history number 8881. White male, aged 41. Onset was sudden. Admitted in the afternoon of January 9, 1929, having worked as usual the previous day. Patient was unconscious. Physical examination was negative except for rigidity of neck and a temperature of 40.0°C. Spinal fluid turbid with 20,800 cells, many diplococci. Blood leucocyte count was 16,400. Patient never recovered consciousness and died following a generalized convulsion on January 11. Blood culture showed many colonies of meningococci.

Case 3. Unit history number 8322. White girl, age 13. Tonsillectomy early in December 1928. Readmitted in a stuporous condition January 12, 1929. Had

Case 8. Unit history number 9303. White male, age 44. Admitted in delirious state February 6, 1929, called the second day of disease. Had sustained severe injury in left frontal region of the head. Reflexes were hyperactive. Kernig's sign positive, Babinski negative. Blood leucocytes were 29,000. He died the following day. Pneumococcus, Type IV, was cultured from blood and spinal fluid.

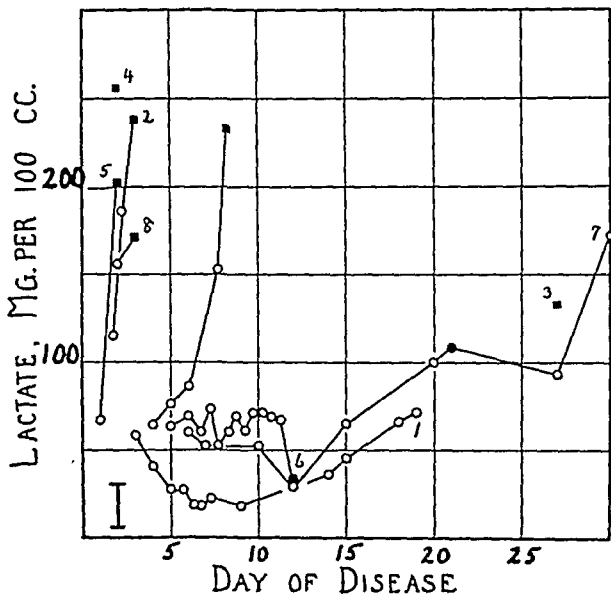


FIG. 3. LACTATE CONTENT OF CEREBROSPINAL FLUID IN MENINGITIS

Symbols as in figure 2

Case 9. Unit history number 9397. White boy, age 8. In latter part of December 1928, he had a gastro-intestinal attack and at this time the right ear commenced to discharge. February 8, ear stopped draining. Admitted February 12, 1929, called the second day of disease. Thigh was flexed on abdomen, subject irritable with rigid neck, positive Kernig's, photophobia, no tenderness over mastoid. Anterior cervical lymph nodes were palpable. Impression: meningitis, secondary to middle ear disease. Lumbar puncture: fluid turbid with 2,700 cells

left. On lumbar puncture, 30 cc. cloudy fluid were removed, at pressure 26 mm. Hg, and containing 17,000 cells per cubic millimeter. Daily administration of antimeningococcic serum was given partly intraspinally and partly intravenously, with apparent mental improvement. The spinal fluid pressure steadily diminished, until by the seventh day when with the pressure at 12 mm. Hg, a little mental retardation was noted. Serum sickness developed. Serum was withheld until the twelfth day, with gradual disappearance of urticarial lesions and less mental apathy. On fifteenth day, he was irritable, head drawn to the left, neck rigid.

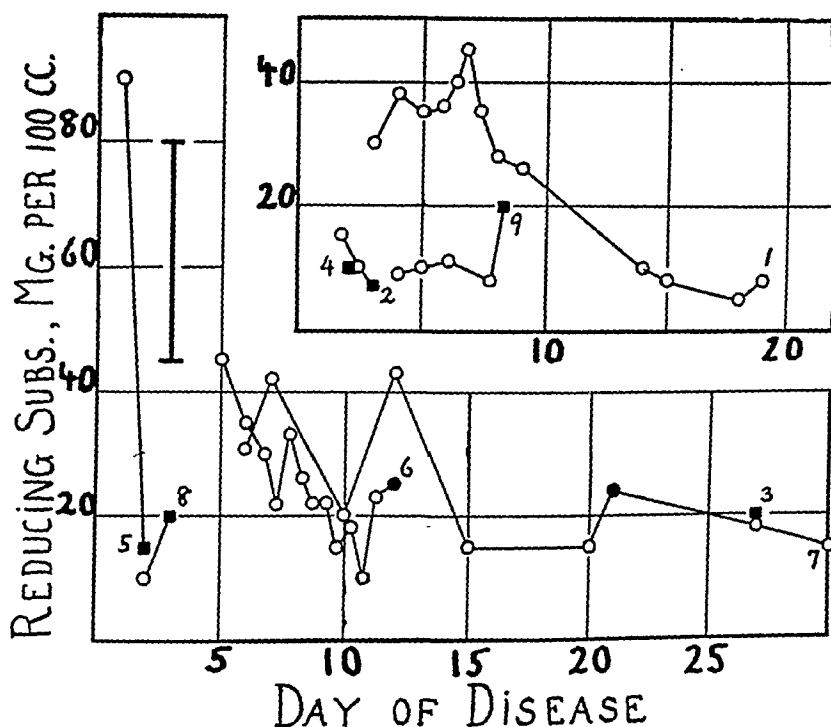


FIG. 2. REDUCING SUBSTANCE IN THE CEREBROSPINAL FLUID IN MENINGITIS

Normal range indicated by vertical line. Case numbers indicated. Other symbols as in figure 1.

fluid pressure 36 mm. Eighteenth day, pressure 56 mm. Hg; he seemed better. On the nineteenth day he asked for food. On the twenty-seventh day the canal was irrigated with saline between cisternal and lumbar punctures. Thirtieth day: pressure 20 mm. Hg, fluid bloody and ran slowly. The respiration became labored and death occurred on March 5th, the thirty-fourth day of disease. Temperature in hospital, 37.2 to 39.4°C. Spinal fluid showed pure culture of meningococcus. Our data include the recovery from serum sickness and the final decline.

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chiefly polynuclear cells, many streptococci, at pressure of 12 mm. Hg. Mastoidectomy was performed on the third day; bloody pus was found in right mastoid. He became progressively worse. On the sixth day antistreptococcal serum was injected; fluid pressure 24 mm. Hg. Died February 18. Temperature in hospital was 39.4 to 40.0°C. Spinal fluid culture showed hemolytic streptococci.

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THE PROPORTION OF CERTAIN IMPORTANT INORGANIC CONSTITUENTS IN THE DYING HEART MUSCLE

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Careful survey of the literature does not reveal many examinations of the cardiac muscle for inorganic elements. As a matter of fact I have only been able to discover one analysis of the human heart which makes any pretension to a detailed series of determinations, by Lematte et al. (1) and this apparently was only on one heart. These investigators determined the percentages of alkali and alkaline earth metals, phosphoric acid, iron, and dry residue, and reported their results in the form of the hydroxides of sodium, potassium, calcium, and magnesium.

Determinations of potassium and sodium have been recently made on human heart and skeletal muscle by Harrison, Pilcher, and Ewing (2). Norn (3) also gives a series of potassium and sodium determinations made on the human dog and ox heart. Matthews (4) presents some figures on the inorganic constituents in his textbook. Aside from these few, efforts to trace other analyses complete or partial have so far failed.

Among these earlier workers who laid the foundation for our present knowledge of physiological action of potassium, sodium and calcium the noteworthy are Howell (5) and Ringer (6). Zwaardemaker (7) has within recent years extended and developed the earlier investigations of Howell and Ringer paying especial attention to the activity of potassium.

That the quantity and proportions of the inorganic constituents of the muscle are vitally important to its activity is self-evident and that within limits they will vary from individual to individual in health and disease is a reasonable assumption. Maxima and minima as well

as optimum proportions have been frequently demonstrated in amphibian hearts by Zwaardemaker and others; the questions arise just what amounts in the human hearts are compatible with life and continuous function, and how do they vary in disease? Qualified answers can only be given in terms of arithmetical means which are equivalent to generalities for the reason that rarely is any death free from contributory causes that make classification at best only an approximation. Nevertheless the determination of the average proportions of the important inorganic elements in diseased cardiac muscle cannot be without interest, and with this object in view the analyses were made.

The results are believed to be accurate and dependable. On fresh hearts a total of 69 analyses were made, eleven however, for water, ash, sodium and potassium only, since the relation of other elements was considered to have been sufficiently well established with the first 58 analyses.

A large number of the alkali determinations were discarded because of probable inaccuracy. The later ones were made with a modified technique, checks having shown these results to be within the limits of experimental error.

Analyses include the determination of water, ash, P_2O_5 , Fe_2O_3 , CaO , MgO , K_2O , and Na_2O . The alkalies are expressed in terms of oxides for the sake of uniformity.

METHOD

Segments of the left ventricle of the hearts were cleared of epicardium and blood vessels and passed through a meat grinder.

Water. Two portions of this ground material were weighed out in beakers. Either 31 or 34 grams were taken in order to obtain from five to seven grams of dry substance. These portions were dried gradually, beginning at about $85^{\circ}C$. and increasing to $100^{\circ}C$. for several days until a relatively constant weight was obtained. Constant weight to better than 5 mgm. was hardly obtainable within a reasonable time, nor was the result appreciably modified by even greater differences. Ammonia salts are probably responsible for this difficulty. Alternate heating and drying in a vacuum was tried with but little better success. The duplicate determinations usually agreed well to the second decimal place.

Ash was determined by igniting 5 or 6 grams and in later determinations 10 grams of dried pulverized muscle in a silica dish at as low a temperature as possible, and keeping the contents just at red heat until white. In this way melting to-

gether of the salts was prevented, and while it is usually impossible to completely free the ash of carbon, the amount is so small as hardly to effect the percentage. The majority of these determinations were made in duplicate with 5 to 6 gram quantities, but in later analyses for potassium and sodium alone with only one portion of 10 grams. The addition of sulphuric acid as suggested by Norn (3) or of nitric acid was not found to be of assistance in destroying organic matter.

Phosphoric acid. The ash from 5 to 6 grams of dried heart muscle tissue was then taken up with strong nitric acid, evaporated to dryness, redissolved in 20 to 25 cc. of 25 per cent nitric acid, 20 cc. of 34 per cent ammonium nitrate solution was added, the contents of the beaker heated to boiling, and the phosphates precipitated with hot 30 per cent ammonium molybdate solution added slowly from a pipette. The precipitate of ammonium phospho-molybdate was filtered off and washed with dilute ammonium nitrate solution. It was then dissolved on the filter with ammonium hydroxide and precipitated with magnesium chloride solution and the P_2O_5 determined from the $Mg_2P_2O_7$ according to the method of Woy described by Treadwell (8). Reduction of the phospho-molybdate and titration with standard permanganate solution gave good results but was not employed as a routine method.

The slight excess of ammonium molybdate appears not to influence any of the later determinations. The removal of the molybdenum is very tedious and the possibility of loss of other elements during the numerous manipulations incident to the removal is in no wise compensated for by subsequent ease in handling the solution.

Iron was estimated in the filtrate from the phosphates by the addition of ammonium hydroxide.

Calcium was precipitated in the filtrate from the ferric hydroxide with a few drops of 15 per cent ammonium oxalate solution. The amount of lime is small and it is necessary to heat for some time with occasional stirring and to wait until the calcium oxalate has completely settled before filtering off. The calcium oxalate was ignited to CaO and weighed as such.

Magnesium is precipitated in the filtrate from the calcium oxalate with slight excess of 15 per cent ammonium phosphate solution. It is necessary to stir very thoroughly until the solution begins to cloud and then to add approximately one fifth volume of strong ammonium hydroxide to insure complete precipitation. Standing over night facilitates this separation. Duplicate determinations almost invariably agree very closely.

Potassium and sodium were determined either by using the ash from 5 to 6 grams of dried material or of that from 10 grams. In both instances solution of the ash was affected with strong HCl , the solution evaporated to dryness, and again dissolved in a few drops of HCl . It was then diluted with water made alkaline with NH_4OH and stirred thoroughly. Milk of lime according to the suggestion of Goto (9) or 25 per cent calcium chloride solution to completely precipitate the phosphates and finally ammonium oxalate to get rid of the excess of lime were added. The beaker was allowed to stand at least 2 to 3 hours, preferably over

night. In this manner all lime, iron, phosphates, calcium, and magnesium are eliminated and nothing remains in solution but ammonium and alkali metal salts.

From this point on the method of Finkner-Neubauer (8) which reference to Neubauer's (10) original work shows to be extremely accurate was used for the determination of potassium and sodium.

When 5 to 6 grams of dried muscle were used for the determination, the filtrate from the precipitate containing the other elements was acidified with sulphuric acid, evaporated and heated to remove excess of acid and ammonium sulphate. The residue was then heated to constant weight using solid ammonium carbonate and the resultant sulphates weighed.

Where 10 gram quantities of dried muscle were employed the procedure was the same except that dilution was first made to 250 cc. in a volumetric flask and 25 cc. quantities equivalent to 1 gram of the original were measured out for each determi-

TABLE 1

Control of method of analysis

Calculated: Sulphates from 0.1 gram NaCl and 0.1 gram KCl = 0.2383 gram; platinum from 0.1 gram KCl = 0.1314 gram

Number	Sulphates	Platinum	KCl	NaCl
	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>
1	0.2370	0.1316	0.1002	0.0986
2	0.2382	0.1290	0.0982	0.1005
3	0.2348	0.1314	0.1000	0.0970
4	0.2378	0.1316	0.1002	0.0992
5	0.2368	0.1316	0.1002	0.0984
6	0.2358	0.1296	0.0987	0.0991
Average.	0.2367	0.1308	0.0996	0.0988

nation. In this way three or more determinations could conveniently be made on each heart.

The mixed sulphates were dissolved in a small amount of water with the addition of a few drops of HCl. This solution should be perfectly clear; turbidity is indicative of incomplete removal of phosphates. Excess of platinum chloride is then added, and the filtration and reduction of the chloro-platinate carried out according to Neubauer. The factors used for the conversion of the platinum into its equivalents were 0.4811 for K_2O , 0.7612 for KCl and 0.8905 for K_2SO_4 .

These manipulations are tedious but have the merit of considerable accuracy. Trials with known quantities of KCl and NaCl gave sufficiently good results. The tables of Neubauer show that when notable quantities of lime, magnesium and sodium salts or excess of platinum chloride, or sulphuric acid, were added to solutions, the estimation of the potassium contained in it was not markedly affected. The method of Kramer and Tisdall (11) determining potassium with

sodium cobaltinitrite or the application of Kramer and Gittleman's (12) gas measurement method employed by Harrison, Pilcher and Ewing (2) is doubtless more convenient but can hardly be more accurate than this old established platinum method.

The series of six consecutive determinations of KCl and NaCl in a mixture of the salts were made without any special precautions and in Pyrex glass vessels as were all those on the cardiac muscle (see table 1).

The main difficulty is encountered with mixed sulphates. The compounds $K_2S_2O_7$ and $Na_2S_2O_7$ said to be produced by the sulphuric acid are not readily converted with ammonium carbonate to neutral sulphates from 0.2 gram of the mixed chlorides but this was very satisfactorily accomplished with the smaller quantities obtained from 1 gram of muscle tissue. There seems to be a tendency for the sodium to be low; the potassium usually checking very well. The potassium and sodium chlorides used were Baker and Adamson C.P. analyzed chemicals.

RESULTS

The hearts on which the determinations were made were utilized as quickly after autopsy as possible. They represent a large variety of diseases, of which those resulting from degenerative changes in the cardiovascular system were in the majority.

An attempt to classify the determinations was made, but owing to the fact that two or more diseases were commonly present at the same time, and the impracticability of working out worth while constants for the group, this arrangement has been abandoned for the present.

Table 2 contains a summary of 58 analyses for water, ash, phosphates, iron, calcium and magnesium, together with the results of 33 and 32 determinations respectively of potassium and sodium.

Another table (table 3) comprises the classification of the analyses according to the racial groups. It will be observed that there is no significant difference between white and colored individuals though the latter predominate. Unfortunately there are only four analyses on hearts from white persons available for comparison of the K_2O and Na_2O contents consequently the standard deviation and probable error have been omitted. Both of these factors have been computed for the other constituents by the usual statistical evaluation methods.

The deviation values are quite large as one would expect from hearts that have been subjected to toxic processes. It is to be deplored that determinations on normal cardiac muscle, at least on hearts of persons not dying of disease are not available for comparison, but it seems quite

probable that even were it possible to always distinguish absolutely normal hearts variability in the constituents would likewise be found.

During the course of the work the percentage of Na_2O was not

TABLE 2
Summary of analyses
(Percentages obtained on the basis of dry muscle tissue. Water excepted)

Constituents	Number of hearts	Mean	Probable error of mean	Standard deviation
		<i>per cent</i>		
H_2O	58	80.130	± 0.139	1.5690
Ash.....	58	4.509	± 0.040	0.4506
P_2O_5	58	1.855	± 0.021	0.2325
Fe_2O_3	58	0.085	± 0.0032	0.0360
CaO	58	0.071	± 0.0035	0.0397
MgO	58	0.137	± 0.0018	0.0209
K_2O	33	1.101	± 0.031	0.2666
Na_2O	32	1.191	± 0.026	0.2145

TABLE 3
Racial grouping
(Percentages on the basis of dry muscle tissue. Water excepted)

Constituents	White				Colored			
	Number of hearts	Mean	Probable error of mean	Standard deviation	Number of hearts	Mean	Probable error of mean	Standard deviation
		<i>per cent</i>				<i>per cent</i>		
H_2O	17	80.090	± 0.207	1.267	52	80.230	± 0.142	1.521
Ash.....	17	4.474	± 0.070	0.429	52	4.564	± 0.044	0.468
P_2O_5	16	1.771	± 0.062	0.168	42	1.888	± 0.025	0.245
Fe_2O_3	16	0.090	± 0.006	0.034	42	0.084	± 0.004	0.037
CaO	16	0.091	± 0.007	0.041	42	0.064	± 0.004	0.039
MgO	16	0.131	± 0.003	0.016	42	0.139	± 0.002	0.020
K_2O	4	0.926			29	1.125	± 0.033	0.264
Na_2O	4	1.380			28	1.164	± 0.026	0.204

and to be in excess of the K_2O . There was no reason of the determinations and the inversion of the often enough to indicate that this is of some force of cardiac contractility. (The proba-

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ble error of the mean in both tables has been calculated, using the formula

$$\frac{0.6745s}{\sqrt{N}}$$

where s is the standard deviation of the observations and N the number of observations.)

Another series of analyses was made on the ventricle and bundle of the ox heart (table 4). The results obtained on the bundle tissue cannot be regarded as better than approximate. The quantity, re-

TABLE 4
Ventricle and bundle of ox heart
(All percentages except water based on dried tissue)

Specimen	Water	Ash	P ₂ O ₅	Fe ₂ O ₃	CaO	MgO	K ₂ O	Na ₂ O
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
<i>Ventricle:</i>								
No. 1.....	79.15	5.301	2.348	0.047	0.013	0.217	1.429	0.843
No. 2.....	78.24	4.842					1.390	1.087
No. 3.....	78.92	5.200					1.479	1.188
<i>Bundle:</i>								
No. 1.....	79.82	3.343					1.205	2.128
No. 2.....	74.89	4.500					1.202	0.928

ardless of care in removal, is small and the chance of drying before proper weighing can be made considerable.

DISCUSSION

In a series of fifty-eight consecutive analyses of fresh hearts' constituents, water, ash, P₂O₅, Fe₂O₃, CaO and MgO have been found to exhibit considerable variation. The water content varies between a maximum of 84.91 per cent and a minimum of 77.21 per cent; the ash between 5.592 per cent and 3.532 per cent; the P₂O₅ between 2.468 and 1.278 per cent; the Fe₂O₃ between 0.153 and 0.030 per cent; the CaO between 0.250 and 0.020 per cent; the MgO between 0.187 and 0.074 per cent. The limits for the values found for K₂O and

Na_2O in 33 analyses are between 1.768 and 0.740 per cent for K_2O and between 1.760 and 0.740 per cent for Na_2O .

The variability is, however, more apparent than real. One has only to examine the columns of figures to gain the impression that there is a tendency to grouping within rather close limits. The least variable of all the constituents is the magnesium and this element is fairly constant regardless of disease. As already stated there appears not to be any correlation between age or disease and any of the constituents. Calcium, which might be expected to exceed in arteriosclerotic changes, is no higher proportionately than in the hearts of individuals dying of carcinoma; nor is age a factor in raising calcium values.

Anyone who has observed a large number of hearts cannot fail to wonder why an organ showing few if any macroscopic or microscopic pathological changes comes to a complete standstill, and, on the other hand, one is equally at a loss to explain how a small or large flabby anemic-looking heart with sclerotic changes or with perforated or stenotic valve flaps can maintain life as long as it does. Questions arise in the mind as to whether or not organic toxic products are entirely responsible for the cessation of cardiac rhythmicity and hence all life processes, or whether perhaps an imbalance between the inorganic constituents exists which is sufficient to cause death. Unfortunately, these analyses do not throw any light on the problem and from this standpoint do not afford the explanation it was hoped they would. Perhaps it is mainly because no accurate determinations of the inorganic constituents in a long series of "normal" hearts exist, such as might be obtained from healthy persons dying as the result of accidents. This would probably be the only means of determining the actual proportions of calcium, magnesium, potassium, and sodium salts indispensable to normal cardiac activity.

Some insight into the rôle played by calcium and potassium in heart tissue has been derived from the work of Ringer (6) and Howell (5) and their collaborators. The meticulous researches of Zwaardemaker (7) on the proportions of potassium necessary to maintain contractility and on the importance of the radioactivity of this element has opened up a new field of research. Furthermore, the investigations of Haberlandt, (13) and Demoor (14) and Zwaardemaker (15) on the "Herzhormon," "substance sensiblatrice" or "automatin," which

according to Zwaardemaker is equivalent to, or at least bears a strong resemblance to, the vitamin B of Eijkman are of interest. All the more is this so as Zwaardemaker has found that this substance is virtually inactive without having been first irradiated by some radioactive substance, or when the perfusing solution contains potassium. According to Zwaardemaker's work, the potassium gives off energy in the form of beta emanation, which is a vital necessity for the maintenance of cardiac rhythmicity.

That calcium, potassium, and sodium are to a greater or lesser degree antagonistic in their action on the cardiac mechanism is abundantly evident from the work of these men. This, of course, leads to the conclusion that for the maintenance of normal conditions there are optimal proportions of calcium, potassium, and sodium. That such an equilibrium must, of course, be maintained in the human heart equally as well as in the hearts of lower animals admits of no argument. It is therefore not impossible that many of the analyses for these necessary inorganic elements would if adequately interpreted reveal a disproportion among these elements incompatible with cardiac function.

Examination of the cardiac tissue, using MacCallum's (16) method of demonstrating potassium *in situ*, was very satisfactory. There is no doubt that the larger proportion of the potassium salts is located within the intercellular spaces.

Employment of MacCallum's stain on sections of the auriculo-ventricular bundle of the ox heart appeared to show less potassium within the cells of the conducting tissue than within muscle cells of the ventricle, but this is uncertain for the moment. There is, however, no doubt that the connective tissue surrounding the conduction fibers was almost entirely lacking in potassium, which is sufficient to account for the lower percentage in the bundle than in the ventricular muscle.

The literature does not disclose any definite reason why magnesium is an invariable and apparently a necessary constituent of cardiac muscle tissue. Like calcium and sodium, it is probably not radioactive and there is no evidence that it is independently active or that it functions as an adjuvant to calcium in promoting tonicity. It does not seem to act as an antagonist to the alkali metals. It is conceivable that its value is that of stabilizer, moderating cardiac hyperirritability.

Na_2O in 33 analyses are between 1.768 and 0.740 per cent for K_2O and between 1.760 and 0.740 per cent for Na_2O .

The variability is, however, more apparent than real. One has only to examine the columns of figures to gain the impression that there is a tendency to grouping within rather close limits. The least variable of all the constituents is the magnesium and this element is fairly constant regardless of disease. As already stated there appears not to be any correlation between age or disease and any of the constituents. Calcium, which might be expected to exceed in arteriosclerotic changes, is no higher proportionately than in the hearts of individuals dying of carcinoma; nor is age a factor in raising calcium values.

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CARBON DIOXIDE AND OXYGEN TENSIONS OF THE MIXED VENOUS BLOOD OF MAN AT REST

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PART I

THE PROBLEM OF EQUILIBRATION OF LUNG GASES WITH INCOMING VENOUS BLOOD

The state of the mixed venous blood, or the blood in the right side of the heart and in the pulmonary artery, especially in respect to its oxygen and carbon dioxide contents, has been of interest since Fick's principle (1) of measuring circulation rate was enunciated in 1870.

In animals the study of this blood can be made directly by puncture of the right side of the heart or by insertion of canulae through the jugular vein into the right auricle. In man such procedures have not usually been considered justified for purposes of experimental study, and recourse has therefore been had to the use of the lungs as an acrotonometer, in the attempt to bring the lung gases into equilibrium with inflowing venous (pulmonary artery) blood before recirculation alters the character of this inflowing blood. Application of these tensions to oxygen or carbon dioxide dissociation curves of the blood then gives the O_2 and CO_2 contents of the mixed venous blood. Numerous methods on this principle have been devised.

Very recently some experiments have been performed with human subjects, in which blood from the right heart was obtained directly. These will be referred to later.

Loewy and von Schrötter (2) in 1905 performed some experiments in which they passed a catheter down the subject's trachea and into one of the large bronchi; then closed off the bronchus by inflating a small rubber bag around the catheter. This portion of the lung thus acted as a closed tonometer. When sufficient time had elapsed in order for

equilibrium to be reached, a sample of the air was withdrawn through the catheter. The sample was supposed to have the same tensions of O_2 and CO_2 as the incoming venous blood. This method assumed that the procedure did not appreciably affect the general circulo-respiratory equilibrium; and it also called for considerable heroism on the part of the subject. It has been little used since.

Four years later Plesch (3) developed the technique of rebreathing gas mixtures in and out of a rubber bag. He attempted to bring the rebreathed mixture into equilibrium both with the O_2 and CO_2 of the venous blood. In order to make the oxygen of the mixture sufficiently low, a preliminary rebreathing of pure nitrogen was employed. Plesch was the first to make the assumption that if, after successive periods of rebreathing, the gases in the bag reached constant values, these values therefore represented equilibrium with the incoming venous blood. We shall return later to a consideration of this assumption.

The measurement in 1914 by Christiansen, Douglas, and Haldane (4) of the effect of oxygenation upon the CO_2 dissociation curves of whole blood led to a further simplification of technique. If a proper mixture of CO_2 and air (or CO_2 and O_2) were rebreathed, it should be possible to oxygenate the incoming blood, without at the same time causing any alteration in its CO_2 content. The CO_2 tension of this blood will, of course, be raised over the true venous level, due to its oxygenation. Blood in this state was called "oxygenated venous," or (Y. Henderson and Prince (8)) "virtual venous" blood. The technique of Christiansen, Douglas, and Haldane was as follows: the subject, after a short preliminary rest period, inhaled from a small rubber bag a mixture of from 6.5 to 10.0 per cent CO_2 in air (sometimes CO_2 in oxygen), held the mixture in the lungs $4\frac{1}{2}$ seconds, then expired about 1 liter, held the breath 6 seconds longer, then made a complete expiration. Samples of the two fractions were analyzed. In some experiments three partial expirations were made, at 4, 9, and 13 seconds. If the CO_2 values of these successive samples were the same, or nearly so, it was believed that equilibrium had been obtained. Further evidence of equilibrium was obtained by having the subject rebreathe successively (a) a mixture which when mixed with alveolar air would give a CO_2 tension higher than the oxygenated mixed venous; and then (b) a mixture giving too low a tension. Thus the equilibrium level was ap-

proached both from above and below. It was assumed here that the lung gases became completely mixed by being simply held in the lungs for $4\frac{1}{2}$ seconds; and also that the equilibrium with the incoming blood could be reached in $4\frac{1}{2}$ (or 9) seconds by the necessary transfer of gases to or from the circulating pulmonary blood. Theoretically, if mixture in the lungs were not perfect, it would have been quite possible for an inhaled mixture that was too low to give in the $4\frac{1}{2}$ second sample a value higher than the completely mixed air, on account of the greater proportion in that sample of air from the bag; while the $10\frac{1}{2}$ second sample might equal this value by increase of CO_2 in it, derived from the blood; without, however, complete equilibrium with the venous blood having been reached at any time. In the cases where successive experiments with different initial mixtures were performed, and good agreement was obtained, this possibility was eliminated.

The results of Christiansen, Douglas, and Haldane showed rather large variations in equilibrium values obtained, between successive experiments, also at times in single experiments agreement of samples was not good. However, the general level of oxygenated mixed venous tension was found, and this marked at the time a signal advance.

The calculations whereby Christiansen, Douglas and Haldane, transferred tensions into terms of blood gas contents were cumbersome, and involved the calculation of the actual mixed venous CO_2 tensions.

Fridericia in 1918 (5) determined venous CO_2 tensions by a technique similar to that of Christiansen, Douglas, and Haldane, except that when differences were found between the tensions obtained at half and full expiration, he calculated the amounts of CO_2 absorbed or given off, and by using varying initial mixtures was able to estimate the tension of zero absorption, or equilibrium. By a method slightly modified from this, Liljestrand and Lindhard in 1920 (6), compared circulation rates as calculated by this and by the Krogh-Lindhard (7) nitrous oxide method, and found satisfactory agreement.

Henderson and Prince (8) in 1917 developed a method for measuring venous tensions similar to that of Plesch with the difference that CO_2 alone was measured. The method consisted in making a deep expiration into a bag, then, after a rest interval of several minutes, inhaling this mixture, holding it in the lungs for 5 to 10 seconds, and exhaling it again; this procedure was repeated several times, and it was found that

a CO_2 tension was reached after 3 or 4 trials which remained constant through further rebreathings. This was considered to be the oxygenated venous CO_2 tension, and it was suggested that the venous CO_2 content could then be obtained by equilibrating a sample of the subject's blood, with this rebreathed air. Henderson and Prince checked their method in two ways: (a) they found that by their method of "intermittent rebreathings" the same final equilibrium tensions were obtained whether they started with a high or low CO_2 tension in the rebreathing bag. They also investigated the effects of holding the air in the lungs (or of rebreathing it slowly) for varying lengths of time, from 3 to 20 seconds or more. Thus, they obtained one equilibrium value, with successive periods of rebreathing, each of 3 seconds' duration, another value after a series of rebreathing, each of 10 seconds' duration, and so forth. They found that between 6 and 16 seconds, a "plateau" level was reached, the equilibrium values obtained for these durations of rebreathing, being essentially the same; these values they interpreted to be the oxygenated venous CO_2 tension. This was a fair inference, except that they did not know what their oxygen tensions were; and it is possible that the transient interruption in a steady rise of venous CO_2 (as represented by the "plateau") was only an apparent equilibrium, due to a falling off of lung O_2 tension with consequent check in the CO_2 tension rise.

Meakins and Davies (9) in 1922 employed a technique similar to that of Henderson and Prince with the difference that the air was regularly rebreathed in and out of the bag twice, instead of simply being held in the lungs. They believed that in this manner, more perfect mixture could be obtained.

Barcroft, Roughton, and Shoji (10) equilibrated the oxygen in the subject's lungs with that in the venous blood, instead of equilibrating the CO_2 . This they did by simply rebreathing pure nitrogen. They found in successive trials that the oxygen tension soon reached a constant level each time at the end of 7 or 8 seconds of rebreathing. This level was maintained till about the twentieth second, after which it decreased rapidly. (This latter fact demonstrated the time of onset of recirculation of blood.) The CO_2 tension at this equilibrium level was lower than that of true mixed venous blood, on account of the

large admixture of nitrogen and very low oxygen tension. Barcroft and his associates recognized this and made certain corrections for it.

At about the same time, Redfield, Bock and Meakins (11) devised an extrapolation method for determining simultaneously both the actual CO_2 and O_2 tensions of mixed venous blood. They rebreathed a given mixture of CO_2 , O_2 and N_2 , and took a sample at the end of 5 seconds and another at the end of 10 seconds. These were analyzed, plotted on a CO_2 - O_2 tension diagram and a line drawn, connecting the two and extrapolated beyond. Similar experiments were done using different initial rebreathed mixtures. It was found that the lines drawn through the various pairs of 5 and 10 second points, when extrapolated, tended to meet at the same point. This was inferred to represent the mixed venous tensions. The procedure assumed that the rate of change of CO_2 , during equilibration, held a constant ratio to that of O_2 . The authors found further that the point of intersection of two or more extrapolated lines was also crossed by a horizontal line drawn at the CO_2 tension as obtained by the Henderson-Prince technique. This last point casts a certain doubt upon the accuracy of the method; because the Henderson-Prince *oxygenated* venous CO_2 tension ought to have been considerably above their own *true* mixed venous CO_2 tension.

Recently Grollman (12) has compared values of cardiac output as obtained by the use of this extrapolation technique, with similar values by the acetylene method, and finds satisfactory agreement.

In 1922 Douglas and Haldane (13) continued further the experiments which Christiansen, Douglas, and Haldane had made in 1914. Knowing the oxygenated and reduced CO_2 dissociation curves and the O_2 dissociation curves of their subjects, these workers were able to find both the oxygenated mixed venous CO_2 levels and the true mixed venous CO_2 and O_2 levels in successive experiments on the same day, and compare the arteriovenous differences (or circulation rates), so obtained. The method used differed slightly from their earlier one. A mixture was made up in a Douglas bag which had approximately the desired concentrations of CO_2 and O_2 (previously determined by a pilot experiment). Three successive complete inspirations were then made from the bag, each inspiration having been preceded by a complete expiration to the outside air. The last inspiration was held

a CO_2 tension was reached after 3 or 4 trials which remained constant through further rebreathings. This was considered to be the oxygenated venous CO_2 tension, and it was suggested that the venous CO_2 content could then be obtained by equilibrating a sample of the subject's blood, with this rebreathed air. Henderson and Prince checked their method in two ways: (a) they found that by their method of "intermittent rebreathings" the same final equilibrium tensions were obtained whether they started with a high or low CO_2 tension in the rebreathing bag. They also investigated the effects of holding the air in the lungs (or of rebreathing it slowly) for varying lengths of time, from 3 to 20 seconds or more. Thus, they obtained one equilibrium value, with successive periods of rebreathing, each of 3 seconds' duration, another value after a series of rebreathing, each of 10 seconds' duration, and so forth. They found that between 6 and 16 seconds, a "plateau" level was reached, the equilibrium values obtained for these durations of rebreathing, being essentially the same; these values they interpreted to be the oxygenated venous CO_2 tension. This was a fair inference, except that they did not know what their oxygen tensions were; and it is possible that the transient interruption in a steady rise of venous CO_2 (as represented by the "plateau") was only an apparent equilibrium, due to a falling off of lung O_2 tension with consequent check in the CO_2 tension rise.

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cient to oxidize the blood completely, and that the CO_2 tension was correspondingly 1 to 3 mm. lower than when $\text{CO}_2\text{--O}_2$ mixtures were rebreathed. They showed also that the same CO_2 level was reached at the end of the second as at the end of the sixth rebreathing, thus indicating a plateau level, from which it was inferred that a state of equilibrium had been reached. The matter of the diluting effect of the residual alveolar air was discussed, as to whether this might prevent a true equilibrium with venous blood being attained. This was tested by adding to the bag 12 cc. of pure CO_2 between rebreathing periods; it was shown in three experiments that the same level was reached in 15–20 seconds, whether the extra CO_2 was added or not. In their further experiments, however, in measuring circulation rates in various individuals the mixed venous values were so variable in some instances, and the circulation rates so large, that the question does arise as to whether a true equilibrium with venous blood can be reached by this method in all subjects.

In further work from the same laboratory, recently reported, (17) this possible source of error has been eliminated. The technique now employed is to have the mixture in the rebreathing bag about 2 or 3 mm. higher in CO_2 content than the expected oxygenated venous level, thus compensating for the diluting effect of the alveolar air. The present authors also, in a recent communication (18), have reported a similar technique.

Field, Bock, Gildea, and Lathrop confirmed Barcroft, Roughton, and Shoji in demonstrating that at rest, the time when evidences of recirculating blood began to be apparent was at the end of about 20 seconds of rebreathing, the CO_2 tension then starting a progressive rise above the plateau level.

The importance of having sufficient oxygen in the rebreathing mixture, in all methods of obtaining oxygenated venous tensions, is stressed in two recent papers by Israëls and Lamb (19).

The most elaborate of all experiments so far reported for arriving at the actual state of the mixed venous blood have been those of Burwell and Robinson (20). These investigators by the use of a double rebreathing system, first washed out the subject's lungs by two respirations from a spirometer containing nitrogen, then completed the equilibration by rebreathing suitable mixtures of CO_2 , O_2 , and N_2 .

They took their sample at the end of about 20 seconds from the time of the first respiration. By employing this technique successively with mixtures that were above and below equilibrium levels, in their initial CO_2 and O_2 tensions, and reaching essentially the same final values with each, they concluded that they had arrived at the true mixed venous tensions. In order to convert tensions into blood gas contents, samples of venous blood were equilibrated in tonometers with the lung air samples obtained at the end of rebreathing.

This method, though laborious, would certainly seem to be as nearly free of theoretical objections as any that has yet been described.

One further point has received special attention recently and that is the question of completeness of mixture of the lung gases,—whether the samples in the rebreathing bag are identical with the air in the pulmonary alveoli. On this subject, Grollman and Marshall (21) have reported some experiments which indicate that only after about 5 rebreathings taking at least 12 to 15 seconds, is such complete mixture obtained. On the other hand, Bock, Dill and Talbott (17) have found that simply holding an inhaled mixture in the lungs for 5 second periods is as efficient in this respect as rebreathing the mixture a greater number of times. Earlier work on this problem was done by Sonne (22).

Hamilton, Moore and Kinsman (23), in a recent paper, have justly criticized the intermittent rebreathing methods, in that in all of these (except where CO_2 is added to the bag between rebreathings), irrespective of what the initial rebreathed mixture is, actual equilibrium will always be arrived at from below, on account of the diluting effect of the residual air in the lungs. They have, however, advanced a further hypothesis, namely, that at any given moment, while the body is at rest, the “blood in the lungs is not wholly venous but more or less arterial in some parts and venous in others, and the tension of the lung air is an expression of the average of these various tensions.” On this hypothesis, they believe that it is possible that the continued rebreathing of a CO_2 — O_2 mixture will only very gradually—in 15 to 20 seconds or more—come into equilibrium with the actual venous (pulmonary artery) blood, being delayed by the arterialized blood in the lungs. We do not see the justification for this latter deduction. Irrespective of how the blood in various individual lung capillaries

may differ in rate of flow, the blood in these capillaries will be exposed at once to pulmonary gases and will presumably come into equilibrium with these gases promptly; whereas all the blood behind this capillary blood will be mixed venous blood, and all blood in front of the capillary blood will be arterialized blood. If the lung and blood tensions are sufficiently close so that lung-blood equilibrium occurs when blood and gas come in contact in the alveoli, then the only way by which a true general equilibrium can be delayed is by incomplete mixing of gases in various parts of the lungs during the first few seconds of re-breathing. If this were the case, there might be some of the alveolar spaces that remained for these few seconds untouched by the re-breathed CO_2 - O_2 mixture. Practically this is not a problem with normal individuals, as by proper rebreathing methods, complete mixture of lung gases can readily be obtained.

The experiments reported by Hamilton, Moore and Kinsman are clear. They used a mixture of 6 per cent of CO_2 in oxygen in a rebreathing bag. Their subject then rebreathed this mixture for six seconds, rested, rebreathed again for six seconds, and so forth until a constant CO_2 tension was found in the bag upon successive rebreathings. The same procedure was employed with 9, 12, 16, and 25 second rebreathing periods. It was found (contrary to Henderson and Prince's work) that no plateau level was reached but that the CO_2 tension increased progressively from the sixth to the twenty-fourth second.

This can readily be explained, it seems to us, by the simple hypothesis that in this subject the diluting effect of the residual air is more than can be made up for by passage of CO_2 from blood into lungs before recirculation occurs. Another set of experiments is also reported, in which CO_2 was added to the bag before each rebreathing. Again no plateau level was found; but here only three rebreathing times were measured, 8, 16, and 24 seconds, so that even if a plateau had existed in the interval from 10 to 20 seconds (where it has usually been reported) the data would not have shown it.

Further evidence of the "partial arterialization" of lung blood, as given by the authors, was that upon rebreathing a CO_2 - O_2 mixture, the total CO_2 in lungs and bag together, appeared to decrease during the first few seconds. This CO_2 , it was argued was absorbed by the

arterialized blood in the lungs. It seems more probable, as suggested in a somewhat different connection several years ago by Christiansen, Douglas, and Haldane (4), that the CO_2 thus lost passes into the tissue fluids of the lung, in raising them from alveolar to oxygenated venous CO_2 tension levels.

Mixed venous values can of course be derived indirectly from cardiac output figures as obtained by foreign-gas inhalation methods, providing the gas contents of the arterial blood, and the minute output of CO_2 or absorption of O_2 , are known. The extensive work that has been done by these methods is outside the scope of the present study.

It is self-evident and well-recognized that all the methods cited above, of obtaining mixed venous blood values, are based on a number of inferences.

The development of a method whereby blood can be drawn from the right heart or pulmonary artery, and compared with "mixed venous" values obtained on the given subject at the same time by one of the indirect methods, will probably be necessary before the absolute accuracy of these methods can be known.

Forssmann (24) recently reported an experiment in which he passed a catheter into his own arm vein, along the subclavian and superior vena cava and so into the right heart. He did not make any blood-gas measurements.

Lauter (25), with Bauman and Friedländer, has investigated the volume output of the heart by actual puncture of the right ventricle and of an artery in man, and has found that the average arteriovenous oxygen difference under basal conditions in normal man is about 5 volumes per cent, and the average volume output of the heart about 4.5 liters per minute. This agrees satisfactorily with the results obtained by several of the indirect methods in the literature cited above. In general, certain foreign-gas inhalation methods appear to give values slightly lower than this, and certain Fick principle methods values somewhat higher; this work has been recently reviewed by Marshall (6).

Even after the direct determination of the CO_2 and O_2 in the blood from the right heart has been made, with the establishment of the accuracy, or the amount of error, of the indirect methods, these latter methods will probably continue to be used in experimental work, as

one would expect that the obtaining of blood from the right heart will remain a somewhat formidable procedure.

It is of interest, therefore, to examine these methods as critically as possible, from the point of view of the assumptions implied in accepting their results.

The following are some of the more important assumptions usually made, explicitly or implicitly, in determinations of mixed venous values.

1. The actual procedure of rebreathing, etc., has no appreciable effect on the values obtained. This is not a simple question to decide. In the methods where several deep respirations are employed within a few seconds, the increased respiratory activity will increase the CO_2 tension and decrease the O_2 tension in the blood coming from these muscles; the "bellows" action of the chest will increase the rate of venous blood flow toward the heart, especially from the abdominal viscera. Whether this latter action will increase or decrease the "venosity" of mixed venous blood will perhaps depend largely on the state of the abdominal (especially liver) blood as compared to other venous blood.

On the other hand, in the methods where the breath is held for several seconds in inspiration, the effect will be to slow the general venous return, and there will be presumably less change in respiratory muscle activity.

The fact (17) that essentially the same mixed venous CO_2 values are obtained whether the breath is held, or continuous rebreathing is employed, suggests that the resultant of the effects of this procedure does not involve a large error,—not more, probably, than 0.5 mm. Such error as there is will be expected to make the CO_2 tension as measured, higher than the actual resting mixed venous CO_2 tension.

2. There is often assumed to be perfect mixture of all gases in the lungs, and between lungs and rebreathing bag. In earlier work this was taken for granted; more recently, Grollman and Marshall (21), also Bock, Dill and Talbott (17) have investigated this point, as noted above. This later work would seem to answer objections based on the possible disturbing effects due to the "fan-like" character of pulmonary expansion.

3. The assumption is made that within the duration of these re-breathings (variously taken from 12 to 28 seconds) there is no appreciable amount of recirculating blood. The argument is made, reasonably enough, that if there were recirculating blood in progressively increasing quantities, there would be no "plateau" of equilibrium; as will be shown more fully later, however, a considerable amount of recirculating blood will cause only a slight upward trend to the CO_2 plateau, and such a trend is present in most of the reported data. Thus there is good reason to suspect recirculation of the coronary blood. This error would make the mixed venous CO_2 tension, as measured, something less than 0.5 mm. above the actual mixed venous value.

4. It is assumed that the rebreathed air and the blood in the lungs are in perfect equilibrium in respect to CO_2 , and in respect to O_2 also when true mixed venous tensions are being studied. When oxygenated venous values are being sought, it is assumed that equilibrium exists in respect to CO_2 and that the blood hemoglobin is completely saturated with oxygen. This last point was demonstrated for the Field, Bock, et al. (16) method in two experiments by them, involving arterial punctures 20 seconds after rebreathing was begun. More recent work (27) from the same laboratory, however, suggests that even with hyperventilation and high alveolar O_2 values, the arterial blood is rarely more than 97 per cent saturated. If this is the case, then the assumption of complete saturation involves a small error; this will make the oxygenated mixed venous CO_2 tension, as measured, lower than the actual oxygenated mixed venous O_2 tension.

The assumption of lung-blood equilibrium, so far as the true mixed venous blood (both a CO_2 and O_2 equilibrium) is concerned, has not previously been tested.

5. It is assumed that with the use of high oxygen mixtures to obtain oxygenated venous CO_2 values, when a plateau of CO_2 tension is reached, this will represent on the CO_2 curve the true level of CO_2 in the venous blood. There is a clear fallacy here, first pointed out to one of us several years ago by Dr. C. D. Murray. As oxygen is continually being absorbed, the total volume of rebreathed air is diminishing and CO_2 , therefore, becoming concentrated, and the final equilibrium value obtained for CO_2 will be above that of the incoming venous

blood. The extent of this effect will be shown by calculation presently to be in the region of 0.5 mm.

It is not improbable that the effects described in paragraphs 4 and 5 tend to neutralize each other.

6. Most of the methods previously employed depend, for the demonstration of equilibrium values, upon successive rebreathing periods, usually four or more within 15 to 30 minutes. They thus assume a steady state in the subject's circulo-respiratory conditions. With trained subjects the constancy of results obtained would seem ample justification for this.

7. All of the methods based on the general technique of Henderson and Prince make the further assumption already referred to; namely that the diluting effect of the residual air in the lungs can always be compensated for by CO_2 given off from the blood in the pulmonary vessels; and, therefore, that when the same value is reached, on successive rebreathings, this value represents the mixed venous level. That such an assumption is unwarranted has already been indicated. With a given constancy of technique, some constant value would always be reached eventually, whether this value had any relation to the mixed venous blood or not. Thus, suppose the following conditions: (a) air in bag 2,000 cc., containing 44 mm. CO_2 ; (b) residual air 1700 cc. containing 35 mm. CO_2 ; (c) oxygenated mixed venous tension 47 mm. CO_2 ; (d) and the amount of CO_2 that can be given off into the lung air from the blood in 20 seconds as 20 cc. A simple calculation will show that when this bag mixture is rebreathed for 20 seconds, the final "equilibrium" value will always be 44 mm. CO_2 in the bag and lungs. (In this calculation, for purposes of simplification the effect of O_2 absorption during the process is neglected.)

By altering the length of time of rebreathing, as Henderson and Prince (8) did, or by changing the mixture used, between rebreathings, as Field, Bock, et al. (16), and Bock, Dill, and Talbott (17) have done, this possible source of error can be investigated. It should be borne in mind, however, that this method may be applicable to some subjects and not to others. The subject of Hamilton, Moore and Kinsman's (23) experiments, for example, was apparently one of the latter. In general, those subjects with large arteriovenous differences, low circulation rates, and large residual air volumes relative to the volumes

of air in the rebreathing bag, would be unfavorable types for these experimental methods.

8. It is a further assumption of all these methods, except those in which blood is drawn at the time of the experiment, and CO_2 and O_2 dissociation curves constructed, that although only gaseous tensions are measured, these can be translated into blood contents of CO_2 or O_2 , by the use of standard dissociation curves.

It is clear, however, that for the purpose of obtaining arteriovenous differences, it is not necessary to suppose that the dissociation curves used are identical with that of the subject at the moment of rebreathing. In the case of oxygenated venous CO_2 tensions, the slopes of the curves used, over the physiological range, must be the same as those of the subject's blood. In the case of true mixed venous tensions, both the slopes of the CO_2 curves and the "spread" (distance between reduced and oxygenated curves) must be the same. These principles have been well recognized in recent years.

In sum, one can perhaps say that of these eight assumptions, those in paragraphs 1 and 3 are the most likely to be incorrect, and the least likely to be detected. The effect of respiratory movement and of recirculating blood would both act to make the mixed venous CO_2 figures higher and circulation rate figures lower than the actual values; and the errors would be essentially the same by both Fick-principle and foreign-gas inhalation methods. The total error involved is probably less than 10 per cent of the arteriovenous difference.

In spite of this number of assumptions, most of the various methods used have given results for mixed venous tensions that are of the same order of magnitude,—an average level of from 45 mm. to 50 mm. of CO_2 tension for most subjects. It is also true, however, that some methods, when used for the determination of circulation rate, give definitely higher values than other methods; some methods also give unexpectedly large variations in circulation rate for different normal individuals. These facts suggest that there may be with some methods a constant error; and that other methods may be applicable to some normal subjects, and not to certain others.

This brings us to a consideration of certain aspects of the problem of the equilibration of lung gases with incoming venous blood, which have thus far received relatively little attention. (a) Some work,

as described, (6, 12, 13) has been done in comparing one method with another; the greater part of this has been in comparisons of Fick-principle with foreign-gas circulation rate methods. (b) There has been almost no work directed toward the determination of the limits of variation in composition of rebreathed mixtures, within which limits an apparent equilibrium with incoming blood can take place. And conversely, there has been a tendency to accept "plateau" or apparent equilibrium values as mixed venous values. (c) There has been relatively little study of the actual process of equilibration during the course of a single rebreathing technique.

Such studies should help to reveal the presence of false equilibrium values, or systematic differences of one method as compared with another.

In the experimental work which forms the second part of the present study, an attempt has been made to do these three things: (a) to compare three different methods, using the same subjects, these methods representing three different principles of equilibration; (b) to follow the course of equilibration by samples taken every few seconds during the procedure; and (c) (providing equilibrium levels are found) to determine the possible limits of composition of effective rebreathing mixtures.

At this point in the discussion, it may make for greater clearness if we endeavor to describe what may be expected to take place in the lungs during the course of this process of equilibration. The major variables in the process are familiar, and it should be possible to arrive at a fairly accurate and complete description.

I. Consider first the situation when "true" mixed venous equilibrium is being investigated; that is, when lung air is brought into equilibrium with both the CO_2 and the O_2 tensions of the incoming venous blood.

Take certain typical values for the various factors concerned, as shown in table 1 (many of these from an actual experiment on subject D. W. R.).

In this table it will be seen that various assumptions, as previously outlined, have been made.

A. In the first instance, suppose that, after the rebreathing of the mixture has been completed in (say) 15 seconds, (a) mixture of gases is complete, and (b) initial tensions have been such that, when mixture

is effected, O_2 and CO_2 tensions in the lung air will be the same as those of the incoming blood.

In the situation as above defined, certain points are to be noted:

1. Equilibrium will have had to be established, not only between lung air and blood, but between lung air and lung tissue fluids as well. Much of this tissue fluid is presumably in equilibrium with residual (alveolar) air. The initial mixture in the bag must, therefore, have had a certain excess of CO_2 and deficit of O_2 , in order to provide for the change of tissue fluid tension from alveolar to venous levels.

2. Equilibrium, even if it is established between lungs and blood, will not remain absolutely perfect because N_2 will tend to diffuse into

TABLE 1

Tensions of respired gases in atmospheric air, lungs, and blood with subject breathing atmospheric air

Volume of residual air in lungs = 1,700 cc.

Volume of air in rebreathing bag = 2,500 cc.

Circulation rate per minute = 4,400 cc.

Barometric pressure = 755.0 mm.

Units	Outside air (dry)	Alveolar air (= residual)	Arterial blood	Mixed venous lung tensions	Mixed venous blood tensions
Oxygen, mm.	158.5	93.0	80.0	40.0	40.0
CO_2 , mm.	0.2	44.6	44.6	49.8	49.8
N_2 , mm.	596.3	569.4	569.4	617.2	569.4
Water, mm.	0.0	48.0	48.0	48.0	48.0
Total, mm.	755.0	755.0	742.0	755.0	707.2

venous blood, thus concentrating the lung air with respect to its other gases; therefore, a little more O_2 and CO_2 will diffuse into the blood to reestablish equilibrium. On account of the small solubility of N_2 gas in blood, this effect is negligible.

3. With the exception just mentioned, a nearly exact correspondence will be expected to exist between lung and blood gases, once equilibrium has been established, until appreciable amounts of blood begin to recirculate.

B. In the next situation to be considered, suppose the same general conditions as under A, with the further feature that a certain fraction of the blood flow has recirculated within the first 4 or 5 seconds, and

continued to do so, without any other fractions of the blood recirculating for an ensuing 10 or 15 seconds. Can a new equilibrium be obtained? In order for this to be so, it will theoretically be necessary (a) for the true venous blood to pass through the lungs unchanged (lungs at mixed venous tensions), (b) for part of this blood to recirculate through the short-circuit path (thus gaining an added increment of CO_2 and losing O_2), (c) for the new mixed venous blood (contaminated by recirculation) to make a further course through the lungs and re-establish a new venous level. Even then the new equilibrium would not be quite complete. There is no use in trying to reason too closely upon this point. It is clear that with recirculation of appreciable amounts of blood, exact plateau or equilibrium levels will not be obtained. Small amounts of blood can readily recirculate, however, without detection by present experimental methods. Thus, simple calculation will show that if there is recirculation of as much as 10 per cent of the minute volume, this will in ordinary conditions at rest, change the "equilibrium" mixed venous value by 0.5 mm. of CO_2 or less.

C. Assume that the air in the bag is such that upon mixture with residual air in the lungs, the gases are not in equilibrium with the incoming blood. The questions that arise are: (a) how nearly, at any instant, will the lung tensions be in equilibrium with the outgoing arterial (i.e., pulmonary vein) blood—i.e., is there a lag in equilibration between lungs and blood? (b) How large a discrepancy can there be at the beginning of rebreathing between mixed lung air, and venous blood, and still have enough CO_2 and O_2 absorbed into or given off from the blood within 15 to 20 seconds so as to reach equilibrium?

(a) The answer to this question could perhaps be predicted on the basis of diffusion rates of gases in pulmonary alveoli, but too little is known of these conditions to admit of even approximate estimates. The question must be decided by experiment. From an experiment which we report in detail in the subsequent part, it seems clear that for both CO_2 and O_2 in the tensions near the mixed venous values, equilibrium between lungs and blood is nearly complete at the end of the twentieth second of rebreathing.

(b) If we suppose, then, on the basis of the above mentioned experiment, that lungs and blood are in approximate equilibrium, it

becomes possible to determine from a blood nomogram the amounts of CO_2 and of O_2 that will be available from the blood, or else the amounts which the blood can be expected to absorb, when the lung gases have not the same tensions as the incoming venous blood.

Supposing the same general circulatory conditions as given above, how much O_2 and CO_2 will be available in 15 or 20 seconds if the mixed lung gases (CO_2 and O_2) at the outset are 5 mm. lower than the venous gases; and how nearly will equilibrium be reached within this time? Conditions at the start will be as in table 2 (column 3 here is the same as column 6 of table 1, column 2 gives the assumed initial lung tensions).

Presumably, equilibrium between lung air and incoming blood in the alveoli will be approached asymptotically. We have not attempted

TABLE 2

Tensions of respired gases in low O_2 - CO_2 rebreathing mixture compared with tensions in normal venous blood

	Initial mixed lung tensions	Mixed venous blood tensions
Oxygen, mm.	35.0	40.0
CO_2 , mm.	44.8	49.8
N_2 , mm.	627.2	569.4
Water, mm.	48.0	48.0
Total, mm.	755.0	707.2

an integral expression for this change, but have assumed the pressure gradient to remain constant over 1 second intervals and in the case of the CO_2 , have calculated the CO_2 tension in the lungs at each particular second by adding to the initial amount of CO_2 in the lungs, the sum of the amounts of this gas contributed from the blood during each of the preceding seconds, then dividing this number by the total volume of lung-bag air and multiplying by the barometric pressure. The difference between the lung tension thus calculated and the mixed venous CO_2 tensions then gives the pressure gradient for that particular second of time.

By this calculation, it is found that at the end of 15 seconds, the CO_2 tension in the lung-bag system will be 47.8 mm., still 2 mm. below the

mixed venous level, and at the end of 20 seconds 48.4 mm., or 1.4 mm. below equilibrium.

For oxygen, the values at the end of 15 and 20 seconds will be even further from equilibrium because the dissociation curve is flatter than the CO_2 dissociation curve, that is, a smaller volume of oxygen is given off from the blood per millimeter of pressure difference, than of CO_2 .

It may be added here parenthetically that a simpler and sufficiently

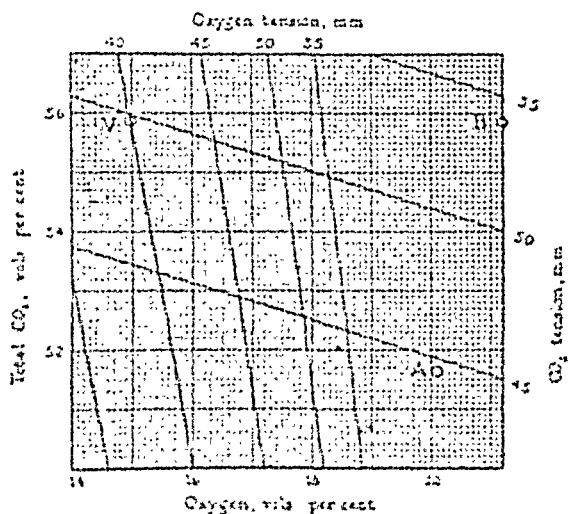


FIG. 1. CO_2 - O_2 Nomogram of Scapier D. W. R.

A, arterial blood, V, mixed venous blood, B, oxygenated mixed venous blood

accurate method for making this calculation is by assuming over the whole 20 seconds of rebreathing a constant average pressure gradient, namely half the initial pressure difference between lung-bag air and blood, or 1.5 mm. for both CO_2 and O_2 . In this region of the nomogram (Fig. 1), 1 mm. change of CO_2 tension at constant oxygen tension corresponds to 0.51 vol. CO_2 (under standard temperature and barometer conditions); and 1 mm. of change of O_2 tension at constant

becomes possible to determine from a blood nomogram the amounts of CO_2 and of O_2 that will be available from the blood, or else the amounts which the blood can be expected to absorb, when the lung gases have not the same tensions as the incoming venous blood.

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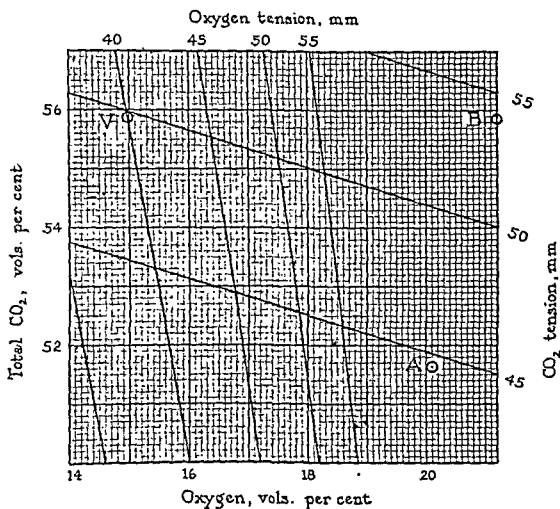


FIG. 1. CO_2 - O_2 NOMOGRAM OF SUBJECT D. W. R.

A, arterial blood; V, mixed venous blood; B, oxygenated mixed venous blood

accurate method for making this calculation is by assuming over the whole 20 seconds of rebreathing a constant average pressure gradient, namely half the initial pressure difference between lung-bag air and blood, or 2.5 mm. for both CO_2 and O_2 . In this region of the nomogram (fig. 1), 1 mm. change of CO_2 tension at constant oxygen tension corresponds to 0.51 cc. CO_2 (under standard temperature and barometer conditions); and 1 mm. of change of O_2 tension at constant

CO₂ tension, to 0.23 cc. O₂. In 20 seconds, there will be $2.5 \times 0.51 \times 20 \times \frac{4400}{60 \times 100} \times \frac{273 + 38}{273} = 21.4$ cc. of CO₂ given off by the blood (at 38°C.) and $2.5 \times 0.23 \times 20 \times \frac{4400}{60 \times 100} \times \frac{273 + 38}{273} = 9.6$ cc. of O₂. The 21.4 cc. of CO₂ would raise the CO₂ tension from 44.8 to $\left\{ \left[\left(\frac{44.8}{755} \times 4200 \right) + 21.4 \right] \div 4231 \right\} \times 755 = 48.4$ mm.; and the 9.6 cc. of O₂ would raise its tension from 35 mm. to $\left\{ \left[\left(\frac{35}{755} \times 4200 \right) + 9.6 \right] \div 4231 \right\} \times 755 = 36.6$ mm.

These estimates are naturally only first approximations, but it is clear that, in order for equilibrium to be obtained by this method between lungs and blood within 20 seconds, the differences in tension between the gas tensions of lungs and blood, at the start of equilibration must be small; less than 5 mm. for CO₂ and considerably less than this for O₂. In the experiments reported in the subsequent part, this conclusion will be verified.

II. In the case where oxygenated mixed venous blood is being studied, a further set of typical values will be useful (table 3).

A. Again we assume here that mixture is complete, and, in the first instance, that the tensions in lungs and bag are such that when mixture has been effected, and incoming blood has been completely oxygenated, then the CO₂ tension in lung air and incoming blood will be the same. In other words, there will be no net change of the CO₂ of the blood during its passage through the lungs. During the first part of the length of the alveolar capillary, while the blood is still partly unsaturated with O₂, there will be a small temporary inflow of CO₂, which will leave the blood again as arterialization is completed. This transient process is here disregarded.

In this instance, however, it becomes necessary to investigate the effect (already referred to above), of O₂ absorption by the blood, and consequent concentration of other lung gases, upon the general equilibrium values.

By reference to the CO₂-O₂ nomogram, figure 1, it is found that the normal venous blood of D. W. R. with an O₂ tension of 40 mm.,

and CO_2 tension of 50 mm., contains 15.0 cc. of O_2 per 100 cc. of blood. The oxygenated blood leaving the lungs after rebreathing the high O_2 mixture will be (it is assumed) completely saturated (Field, Bock, et al. (16)), and will in this case contain 21.2 cc. of O_2 per 100 cc. of blood. About 6.2 cc. of O_2 will, therefore, be absorbed per 100 cc. of blood flowing. In 20 seconds, with a minute volume of circulation of 4400 cc. this will amount to $44 \times \frac{20}{60} \times 6.2 = 91$ cc. of O_2 absorbed. This latter figure represents O_2 under standard temperature and bar-

TABLE 3

Comparison of tensions of respired gases in (a) atmospheric (outside) air, (b) alveolar air, (c) arterial blood, (d) and (e) lungs and blood in "oxygenated mixed venous" equilibrium

Volume of residual air in lungs = 1,700 cc.

Volume of air in rebreathing bag = 2,500 cc.

Circulation rate per minute = 4,400 cc.

Barometric pressure = 755 mm.

	Outside air (dry)	Alveolar air (= residual)	Arterial blood	Oxygen- ated mixed venous lung tensions	Oxygen- ated mixed venous blood tensions
Oxygen, mm.....	158.5	93.0	80.0	100+	100±
CO_2 , mm.....	0.2	44.6	44.6	54.0	54.0
N_2 , mm.....	596.3	569.4	569.4	553-	553±
Water, mm.....	0.0	48.0	48.0	48.0	48.0
Total, mm.....	755.0	755.0	742.0	755.0	755(?)

ometric conditions: $91 \text{ cc.} \times \frac{273 + 38}{273} = 104$ cc. of lung-bag oxygen absorbed. This will change the CO_2 tension in lung-bag system from its initial value of $\frac{300}{4200} \times 755 = 54.0$ mm. to $\frac{300}{4096} \times 755 = 55.3$ mm.

The ultimate effect will be less than this, because the extra CO_2 will redistribute itself between lungs and blood, and much of it, therefore, will be carried away in the blood as fast as it accumulates. As an approximation, we may suppose an average CO_2 tension gradient between lungs and blood (due to oxygen absorption), of $\frac{55.3 - 54.0}{2} =$

0.65 mm. Then in 20 seconds, $0.65 \times 0.51 \times \frac{4400}{300} = 4.9$ cc. of CO_2 reabsorbed. Thus the final lung-bag tension of CO_2 at the end of 20 seconds will be approximately $\frac{300 - 4.9}{4096} \times 755 = 54.4$ mm.

In summary, therefore, this concentration effect is not quite negligible; it should cause the oxygenated mixed venous tensions of CO_2 , at the end of 20 seconds rebreathing, to be in the region of 0.4 mm. higher than the true equilibrium value; and it should further provide that, even if this true equilibrium value is attained at any moment, the CO_2 tension will thereafter show a small steady rise, instead of remaining perfectly constant. This latter effect would be small, however, and by itself would probably escape detection with present methods of analysis.

If, as suggested above, the oxygenated mixed venous blood is actually not more than 97 per cent saturated, it can readily be seen that the error involved in assuming complete oxygenation will tend to counteract the error due to neglecting the oxygen absorption effect.

B. Assume in this instance that lung-bag air (after mixture) is such that upon oxygenation of the blood, the tension of the CO_2 in the blood thus oxygenated is *less* than the CO_2 tension in lung-bag air. CO_2 will pass from lungs into blood. The question arises, how much of an excess of CO_2 can exist in lung-bag air, and still reach an equilibrium with the incoming venous blood at the end of 20 seconds? How much extra CO_2 can the blood absorb?

The attempt to calculate how much CO_2 will be absorbed into the blood, with a given CO_2 tension difference between blood and lung-bag air, raises the further question as to how complete is the equilibrium between lungs and (oxygenated) alveolar blood, with respect to CO_2 . In the region of the tensions of oxygenated mixed venous blood this equilibrium has been shown by Field, Bock, et al. (16), to be nearly complete; rebreathed air samples having been compared with simultaneously drawn arterial blood. It is, therefore, again possible to determine from a blood nomogram, or from a CO_2 dissociation curve of arterial blood, approximately the amounts of CO_2 that can be absorbed in a given interval.

In this calculation account must be taken of the oxygen absorbed

from lung-bag air during the interval. Taking the same basic figures, we have 104 cc. of O_2 absorbed in 20 seconds, out of a total of lung-bag volume of 4200 cc.

Suppose that the lung-bag mixture is 2 mm. higher in CO_2 tension than that of the oxygenated mixed venous blood. Take the same figures as before for volumes, blood gas tensions, blood flow, etc.

At the start of rebreathing, the lungs will contain $\frac{56}{755} \times 4200 = 312$ cc. of CO_2 . In 20 seconds $\frac{4400}{3}$ cc. of blood will flow. For every 1 mm. change in CO_2 tension, 0.46 cc. of CO_2 will be absorbed per 100 cc. blood (see nomogram, from 50 mm. to 55 mm. CO_2 tension on completely oxygenated line). With an average pressure head of 1.5 mm. the CO_2 absorbed in 20 seconds will be $\frac{4400}{300} \times 0.46 \times 1.5 = 10.1$ cc. $312 - 10.1 = 302$ cc. of CO_2 remaining in lung-bag air. The volume of this air will now be $4200 - 104 - 10.1 = 4086$ because of loss of O_2 and CO_2 . The final CO_2 tension will, therefore, be $\frac{302}{4086} \times 755 = 55.8$ mm. In other words, even with the small excess of 2 mm. of lung-bag CO_2 tension, equilibrium cannot theoretically be reached with the incoming venous blood, within 20 seconds. This calculation is admittedly approximate and several small corrections that might be made have been omitted, but the tentative conclusion that it leads to seems unmistakable.

A similar calculation for an excess of 5 mm. in lung-bag mixture (initial CO_2 tension of 59 mm.) leads to a final CO_2 tension (at the end of 20 seconds) of 57 mm.; and an initial excess of 10 mm. of CO_2 (64 mm. tension) gives a tension after 20 seconds of 58.5 mm. Thus it is evident that with increasing excesses of CO_2 in the lung-bag air, the final values at the end of rebreathing steadily, though quite gradually, rise above the tensions of CO_2 of oxygenated mixed venous blood. This type of change will be apparent in the experimental data given later.

It should be noted that in the preceding comments no account has been taken of the buffering effect, so to speak, of the lung tissues themselves. These tissues will, for the most part, presumably be near the gaseous tensions of the alveolar air, and will, therefore, absorb certain

amounts of CO_2 when equilibrium with mixed venous tensions is being approached. It would be difficult to assign any values to the amounts of CO_2 thus absorbed.

C. Finally, it is necessary to consider the case in which the lung-bag mixture is lower in initial CO_2 tension than the oxygenated mixed venous blood. As pointed out above, most of the methods used hitherto, and all of those based on the Henderson-Prince technique, are in this class, the equilibrium values for CO_2 tension being approached from below. What is the lowest initial CO_2 tension that may be used and still establish equilibrium with the incoming venous blood before recirculation?

Again let us take the same physiological values as before. Suppose the lung-bag mixture to be 4 mm. lower in CO_2 tension than the oxygenated mixed venous tension, that is, 50 mm. At the start, in the lung-bag space there will be $\frac{50}{755} \times 4200 = 278$ cc. CO_2 . In 20 seconds approximately $\frac{54 - 50}{2} \times \frac{4400}{300} \times 0.46 = 13.5$ cc. of CO_2 (standard conditions) will be given off from the blood; this at lung temperature will be about $\frac{311}{273} \times 13.5 = 15.4$ cc. The CO_2 tension in the lung-bag mixture after rebreathing will, therefore, be $\frac{278 + 15.4}{4200 - 104 + 15.4} \times 755 = 53.9$ mm. This is practically the actual oxygenated mixed venous CO_2 tension.

If the initial lung-bag mixture were 6 mm. too low in CO_2 tension, then the value after rebreathing would be 53.2 mm., or 0.8 mm. below equilibrium value.

Again no account has been taken of the effect of the lung tissues, which will require certain extra amounts of CO_2 to raise them to oxygenated mixed venous levels.

Thus, under the conditions here postulated, the lowest possible value of initial lung-bag CO_2 tension, that can be equilibrated with the venous blood within 20 seconds will be a tension somewhat less than 4 mm. below the oxygenated mixed venous tension.

Now apply this same type of calculation to an experimental method

in which successive rebreathing periods are employed. Supposing that following a certain period of rebreathing, an equilibrium with the oxygenated mixed venous blood is reached, with respect to CO_2 ; in the next period will the dilution due to residual alveolar air be made up by CO_2 given off from the blood?

Take for the purpose a series of actual data from subject D. W. R.:

Alveolar CO_2 tension 43.9 mm.

Oxygenated mixed venous CO_2 tension 53.3 mm.

Residual air 2,000 cc. containing $\frac{43.9}{755} \times 2,000 = 116$ cc. CO_2 .

Air in bag 2,500 cc. containing $\frac{53.3}{755} \times 2,500 = 176$ cc. CO_2 .

Lung-bag tension at start of rebreathing $\frac{116 + 176}{2,000 + 2,500} \times 755 = 49.0$ mm. CO_2 .

Circulation rate per minute = 5100 cc. per minute.

CO_2 available from blood (see above):

$$\frac{53.3 - 49.0}{2} \times \frac{1700}{100} \times 0.46 \times \frac{311}{273} = 19.2 \text{ cc. in 20 seconds.}$$

$$14.4 \text{ cc. in 15 seconds.}$$

Total air in lung-bag system after 20 seconds:

$$4500 + 19.2 - 120 = 4399 \text{ cc. (120 cc. of } \text{O}_2 \text{ absorbed in 20 seconds).}$$

$$\text{After 15 seconds, } 4500 + 14.4 - 90 = 4424.$$

Final CO_2 tension after rebreathing:

$$\frac{292 + 19.2}{4399} \times 755 = 53.4 \text{ mm. at end of 20 seconds.}$$

$$\frac{292 + 14.4}{4424} \times 755 = 52.4 \text{ mm. at end of 15 seconds.}$$

In this case, then, there is just enough CO_2 available to return the tension to its former value after 20 seconds' rebreathing, providing no account is taken of absorption of CO_2 by lung tissue fluids. If this is appreciable, then the values of CO_2 reached by successive rebreathings for 20 seconds will not represent the actual mixed venous level. Still further deviations will occur if (a) the residual air is greater in volume relative to the air in the bag, (b) blood flow is slower, and (c) arterio-venous difference is greater. It will, therefore, be clear that for a technique of this sort, the actual mixed venous value may be obtained with one subject and not obtained with another.

SUMMARY

Preliminary to some experimental studies upon the mixed venous blood values of man at rest, the previous literature has been reviewed, with special emphasis upon the inferential nature of the results obtained, and the numerous assumptions involved.

Certain aspects of the process of equilibration of lung gases with incoming venous blood, are discussed.

PART II

EXPERIMENTAL

In the first part of the present investigation we have reviewed the previous work on this subject, discussed some of the assumptions upon which the measurements of mixed venous blood in man are based, and described what we conceived to be the probable sequence of events within the lungs when the rebreathing procedures were carried out under various conditions.

In our experimental work, we have attempted to study further the process whereby equilibrium between lung air and incoming venous blood is reached; specifically to find answers to the following questions:

1. Is a "plateau" or apparent equilibrium level in rebreathed lung gases demonstrable over an appreciable length of time, before evidence of recirculating blood is manifest?
2. If such an apparent equilibrium between respired gases and incoming blood is reached, how soon after beginning rebreathing can this be demonstrated, how long does it last, how soon do signs of recirculation of blood appear and how rapidly does this recirculating blood change the apparent equilibrium values?
3. What are the upper and lower limits of respired air mixtures, within which limits an apparent equilibrium can be reached before recirculation of blood occurs?
4. Are oxygenated mixed venous values comparable with actual or true mixed venous values?
5. Do the methods of intermittent rebreathings (without changing the gas in the bag between periods) give the same values as methods using only a single rebreathing period?

6. Are lungs and arterial blood in equilibrium during the rebreathing procedures (only a partial answer attempted)?

The experimental studies which we have made fall under three general headings:

(a) The equilibrium in the case of oxygenated mixed venous tensions, during a single rebreathing period.

(b) The same, studied during intermittent rebreathing periods, with intervals of rest between, and without changing the gases in the rebreathing bag in these intervals.

(c) The equilibrium in the case of the actual mixed venous tensions (equilibrated both with CO_2 and O_2), during a single rebreathing period. Each of these determinations was always either preceded or followed by an equilibration with oxygenated tensions (group (a) above), for purposes of comparison.

Two normal subjects were used, M. L. S. and D. W. R., a considerable number of experiments having been performed with the latter, and a smaller number—with the major purpose of confirming the general findings on D. W. R.—with the former subject. A few relevant data regarding the two subjects are listed in tables 4 and 5.

It is evident that for the purposes of this study it was important to have as many observations as possible taken during the course of a single rebreathing period; this was done by the use of a series of evacuated gas sampling tubes, either six or nine being used to give the corresponding number of separate samples during the experimental period of from 25 to 50 seconds.

METHODS

In further detail, the methods used were as follows:

(a) For oxygenated mixed venous tensions, the simple apparatus shown in figure 2 was employed. Most of the experiments were done toward the end of the morning, the subject having had an ordinary breakfast three or four hours before. A few were done in mid-afternoon, two or three hours after lunch. After 15 minutes to half an hour of rest in the horizontal position, the subject applied mouthpiece and noseclip and respired through the apparatus for five to ten minutes. At the end of this time, at the signal "Blow," given at the end of a normal expiration, the subject expired completely while slide valve B was closed off. At the end of this expiration an alveolar air sample was (usually) taken into one of the sampling tubes. After a rest interval of three minutes, a similar complete expiration was

Subject D. W. R., male, aged thirty-three, height, 188 cm.; weight, 75 kgm.; vital capacity, 6300 cc.; residual air, 2000 cc.

TABLE 4

Oxygenated mixed venous CO₂ tensions

Date	Initial P _{co₂} in bag	Alveolar P _{co₂}	5-second sample	10-second sample	15-second sample	20-second sample	25-second sample	30-second sample	35-second sample	40-second sample	45-second sample	50-second sample
Group I (50 to 56 mm.):												
November 20.....	52.5	48.6	+2.3	50.9	+1.3	52.2	+1.1	53.3	+0.9	54.2	+0.7	54.9 (55.0)
December 3.....	55.8		52.5		(18)	53.3	+1.4	54.7	+0.7	55.4 (55.0)		
December 3.....	55.1		51.7		53.0							
December 7.....	55.9		52.7	0.0	52.7	+1.5	54.2	+1.5	55.7	+0.2	55.2	55.5
December 17, a.m....	56.0		51.8	+0.1	51.9	+1.0	52.9	+1.0	53.9	+1.2	55.1	55.9 (55.6)
December 17, p.m....	53.6		51.8									
December 18, a.m....	54.0		52.3	+0.5	52.8	+1.0	53.8	+1.0	54.8	+0.3	54.9	54.7 (54.7)
December 21, a.m....	51.0	44.1			51.1	+0.7	51.8	+0.8	52.6	+0.4	53.0	55.9 (55.3)
December 26, a.m....	54.2	43.0			51.3	+0.6	51.9	+0.5	52.4	+0.6	53.0	56.2 (55.9)
February 18, a.m....	51.7	45.8			52.2	+0.9	53.1	+0.7	53.8	+0.9	54.7	53.5 (53.4)
	52.5	41.3			49.6	+0.9	50.5	-0.2	50.3	+1.2	51.5	53.8 (53.0)
Average.....	53.8	43.6			51.7	+1.0	52.8	+0.9	53.7	+0.7	54.4	55.2 (54.8)
Group II (56 to 60 mm.):												
December 10.....	56.7											
December 10.....	56.6		53.0	+0.6	53.6	+0.2	53.8	+1.3	55.1	+0.7	55.8 (55.2)	
			52.3	+0.6	52.9	-0.1	52.8	+1.4	54.2	+0.5	54.7	55.3 (55.0)

[illegible]

* Columns marked Δ show the change between successive 5-second samples.

MIXED VENOUS BLOOD OF MAN

made at the "Blow" signal, the slide valve shut down as before, and the 3 way valve turned so as to connect the rebreathing bag with the subject. The subject then emptied and filled the bag completely, by successive respirations, samples being taken into the sampling tubes every 5 seconds at the end of a complete expiration. At first a complete respiration was made every $2\frac{1}{2}$ seconds (subject timing his breathing with the aid of a stop watch, starting the watch at the "Blow" signal). The ten second sample was taken after the third complete respiration, the 15 second sample after the fifth, etc. It was later found that as rapid and complete a mixture of rebreathed gases could apparently be obtained by respiring only once in 5 seconds, holding the air in the lungs for about 2 seconds following each inspiration. At the end of the expiration when the last sample was taken, valve E was turned again, thus shutting off the air in the bag, and the experiment was ended.

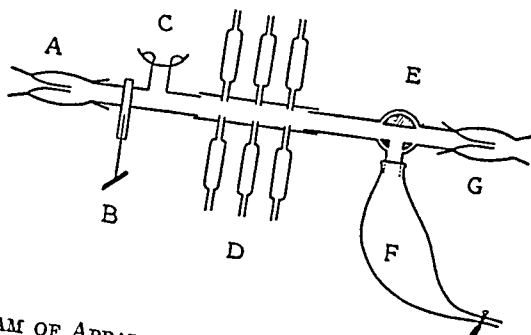


FIG. 2. DIAGRAM OF APPARATUS USED FOR OXYGENATED MIXED VENOUS TENSION DETERMINATIONS

A, intake flutter valve; B, shut-off slide valve; C, mouthpiece; D, six evacuated gas sampling tubes; E, three-way valve; F, rebreathing bag, containing $\text{CO}_2\text{--O}_2$ mixture; G, outgo flutter valve.

A sample of the bag air was taken, immediately, to compare with that in the last evacuated sampling tube.

Usually two such experiments were done in succession, the subject remaining at rest in the 10 to 15 minute interval between experiments. In some of the earlier experiments the subject did not remain at rest, but got up and moved about doing ordinary laboratory work.

(b) In the case of the experiments done with successive rebreathing periods the apparatus was the same, but the technique of collection differed slightly; at the end of a normal respiration, as before, the signal was given, the subject expired completely, slide valve B was closed, and an alveolar sample taken. After three minutes rest, breathing through the apparatus, the same technique was repeated, except that after slide valve B was closed, valve E was turned to connect bag and subject. The subject then rebreathed the gas mixture four times in about 18

seconds. At the end of the fourth expiration, one sample was taken into an evacuated tube, valve E turned again to connect patient with outgo valve, and shut-off valve B opened. The subject rested another 3 to 5 minutes (mouthpiece and noseclip still in place). Then another similar rebreathing was done, as described. This process was repeated every 3 to 5 minutes for five to six periods.

Following this experiment, after a further 10 to 15 minute rest, a single-period experiment (as in (a) above) was carried out, for comparison.

Samples were analyzed for CO_2 with the Haldane apparatus.

The rebreathing bag contained 2.5 liters, the mixture consisting of oxygen plus varying amounts of CO_2 . In order to determine the limits for initial CO_2 tension

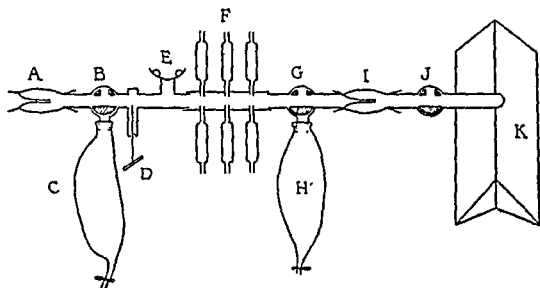


FIG. 3. DIAGRAM OF APPARATUS USED FOR TRUE MIXED VENOUS TENSION DETERMINATIONS

A, intake flutter valve; B, three-way valve; C, rebreathing bag, containing preliminary CO_2 - N_2 mixture; D, shut-off slide valve; E, mouthpiece; F, six evacuated gas sampling tubes; G, three-way valve; H, rebreathing bag, containing CO_2 - N_2 - O_2 mixture; I, outgo flutter valve; J, three-way valve; K, Douglas bag for collection of expired air.

in the bag, within which limits an apparent equilibrium could be obtained by rebreathing, widely different initial tensions were employed, varying from 45 mm. to 80 mm., as shown in tables 4 and 5.

(c) For the determination of actual mixed venous tensions, a slightly modified apparatus was used, based on the technique described by Burwell and Robinson (20). A diagram of the apparatus is given in figure 3.

Bag C was filled with nitrogen plus a small amount—from 30 mm. to 50 mm.—of CO_2 ; this mixture was rebreathed twice, for the purpose of lowering the O_2 tension in the residual air. Bag H was filled with a mixture containing about 55 mm. CO_2 , 35 mm. O_2 , and the rest nitrogen. This latter mixture was worked out, partly by calculation and partly by trial and error, so as to make the resultant gas

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made at the "Blow" signal, the slide valve shut down as before, and the 3 way valve turned so as to connect the rebreathing bag with the subject. The subject then emptied and filled the bag completely, by successive respirations, samples being taken into the sampling tubes every 5 seconds at the end of a complete expiration. At first a complete respiration was made every $2\frac{1}{2}$ seconds (subject timing his breathing with the aid of a stop watch, starting the watch at the "Blow" signal). The ten second sample was taken after the third complete respiration, the 15 second sample after the fifth, etc. It was later found that as rapid and complete a mixture of rebreathed gases could apparently be obtained by respiring only once in 5 seconds, holding the air in the lungs for about 2 seconds following each inspiration. At the end of the expiration when the last sample was taken, valve E was turned again, thus shutting off the air in the bag, and the experiment was ended.

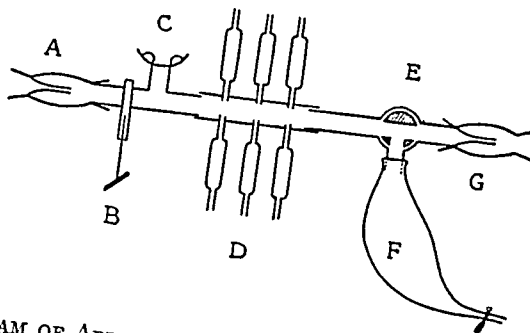


FIG. 2. DIAGRAM OF APPARATUS USED FOR OXYGENATED MIXED VENOUS TENSION DETERMINATIONS

A, intake flutter valve; B, shut-off slide valve; C, mouthpiece; D, six evacuated gas sampling tubes; E, three-way valve; F, rebreathing bag, containing $\text{CO}_2\text{--O}_2$ mixture; G, outgo flutter valve.

A sample of the bag air was taken, immediately, to compare with that in the last evacuated sampling tube.

Usually two such experiments were done in succession, the subject remaining at rest in the 10 to 15 minute interval between experiments. In some of the earlier experiments the subject did not remain at rest, but got up and moved about doing ordinary laboratory work.

(b) In the case of the experiments done with successive rebreathing periods the apparatus was the same, but the technique of collection differed slightly; at the end of a normal respiration, as before, the signal was given, the subject expired completely, slide valve B was closed, and an alveolar sample taken. After three minutes rest, breathing through the apparatus, the same technique was repeated, except that after slide valve B was closed, valve E was turned to connect bag and subject. The subject then rebreathed the gas mixture four times in about 18

give the CO_2 tensions in the fifth-, tenth-, fifteenth-, etc., second samples, and the intervening columns (fifth, seventh, etc.) give the CO_2 tension differences between these successive 5-second values. The figures in parentheses are the CO_2 tensions of samples taken from the rebreathing bag after the procedure was ended; these in general are close to the values of the last of the samples taken in the evacuated sampling tubes.

The experiments are listed in five groups, according to the initial CO_2 tensions in the rebreathing bag; Group I, 50 to 56 mm.; Group II, 56 to 60 mm.; Group III, 60 to 65 mm.; Group IV, 65 to 70 mm.; Group V, 70 to 80 mm. The separation of Groups I and II at 56 mm. is made because of the well defined difference that occurs in the series below this value as compared with the series above.

Averages of all figures are given at the end of each group.

In figure 4, these averages for subject D. W. R. are presented in graphic form. It will be noted that there is in this figure an ordinate marked "Estimated mixed CO_2 tension." This is an estimate of the concentration of CO_2 in the mixed respired gases, calculated from the volume of bag air, its CO_2 content, the volume of residual air,¹ and its CO_2 content. It is assumed that no absorption or elimination of gases by the blood has taken place. The resultant figure gives an approximate measure of the extent of change required for the establishment of equilibrium between lungs and incoming venous blood.

Considering for the moment only the experiments on D. W. R., one finds certain points brought out:

1. In the group where the initial bag tensions were 50 to 56 mm. it is clear that no consistent "plateau" level is reached at any time. There is thus no evidence that an equilibrium has been achieved between lung gases and incoming venous blood. There are considerable differences of tension between successive sets of samples taken at

¹ The subject's residual air was calculated by having him expire completely, then rebreathe from a spirometer a high O_2 mixture several times in 15 seconds, (to obtain complete mixing), then again expire completely into the spirometer. The nitrogen in residual air was assumed to be 75 per cent of the total gas volume (25 per cent taken by CO_2 , O_2 , and water vapor). From this value, the volume of the gases in the spirometer at the end of rebreathing, and the percentages of N_2 in the spirometer at the beginning and end of rebreathing, the approximate value of the residual air can readily be calculated. For subject D. W. R. this volume was about 2000 cc.

tensions, after mixture by rebreathing, as nearly as possible the same as those of the incoming venous blood; i.e., so as to reduce the necessary equilibration to a minimum.

The technique of these experiments, in further detail, was as follows: After the preliminary rest period, and the five to ten minutes of breathing through the apparatus, the signal "Blow" was given, as before, at the end of a normal expiration, and a complete expiration made. Valve B was then opened from bag C to the subject, and valve J closed off. Two complete respirations were made into bag C. Slide valve D was then closed and valve G opened between bag H and the subject. Complete respirations were then made every 5 seconds, a sample being taken into one of the sampling tubes at the end of each, and the exact second of taking noted. The stopwatch was started at the signal "Blow." When the last sample was taken, at the end of about thirty seconds, valve G was closed to the bag and the experiment ended.

The subject was usually moderately cyanosed and dyspneic following these experiments.

After a fifteen to twenty minute rest period, a set of oxygenated mixed venous tensions was obtained as described under (a) above. On one occasion the order was reversed, the oxygenated experiment being done first. No difference was noted.

In certain experiments, for the purpose of obtaining the respiratory quotient, a 5 or 6 minute volume of expired air was collected in a Douglas bag, before the rebreathing was begun, and analyzed for O_2 and CO_2 . A sample of room air was usually also analyzed, as the intake valve did not lead in from the outside air.

RESULTS

(a) *Oxygenated mixed venous blood tensions*

In tables 4 and 5 are given the results, according to the technique described above, of forty-six consecutive experiments with subject D. W. R., and of ten experiments with subject M. L. S. In the case of the former subject this represents every experiment that was done except for two preliminary ones with a slightly different technique. In the case of M. L. S., one day's experiments were discarded because of leaks discovered in the sampling tubes. Every CO_2 determination that was made is also given, but certain values which are enclosed in brackets, are not included in the further calculations, as being improbably far from other values.

In the tables, it will be noted that the second column gives the CO_2 tensions in the bag before rebreathing was begun, and the next column the CO_2 tension of the alveolar air. The fourth, sixth, etc. columns

± 0.3 mm. As the error of method in the Haldane gas analyses is itself about 0.2 mm., this degree of variation can be considered small. Thus there is found at the plateau level both a decrease in the amount of average change in level over the interval, as compared with earlier and later intervals, and a decrease in the deviations of individual values from the average, when compared with similar deviations for the differences in other time intervals.

Even over this plateau, however, it is clear that there is a small rise in CO_2 tension in most instances, with an average change of $+0.19$ mm. CO_2 . This may be due to the recirculation of increasing small amounts of blood, or it may be in part, at least, an effect due to oxygen absorption, discussed in the previous part. In either case, the plateau level should then be slightly above the actual oxygenated mixed venous level.

3. Beginning at 25 seconds, there is a progressive rise in CO_2 level, presumably due to increasing amounts of recirculating blood.

4. The average CO_2 tension level in the three groups is nearly the same, being (at the fifteenth second) 53.0 mm. for Group II, 53.7 mm. for Group III, and 53.3 mm. for Group IV. The fact that with these considerable variations in initial bag mixture, the plateau level is nearly the same is further evidence that this plateau represents a true equilibrium condition.

5. Although average plateau levels are nearly the same, individual experiments show considerable variations in this respect. The lowest figure at 15 seconds is 52.6 mm., and the highest 55.9 mm. Such variations are to be expected. The work of Higgins (29) and later of Dodds (30) on alveolar air values, during the course of the day, and that of Cullen and Earle (31) more recently, on the arterial pH , and CO_2 tensions, show that considerable variations take place, and it is reasonable to suppose similar changes in mixed venous levels. There are, in fact, some early experiments by Porges, Leimdörfer, and Markovici (32), using the original Plesch method, which showed such variations in mixed venous tensions. It is clear, for instance, from our data, that the mixed venous tension level is usually higher in the afternoons than in the late mornings. A further point, which is obvious enough but easy to lose sight of, is that these data represent

5 second intervals. (On the chart there is an apparent equilibrium from 10 to 15 seconds, but reference to the table will show that this is false, and due to the fact that for certain experiments no 10-second sample was obtained.)

2. In the next three groups, with initial tensions at 56 to 60 mm., 60 to 65 mm., and 65 to 70 mm., there is a clear cut "plateau" found

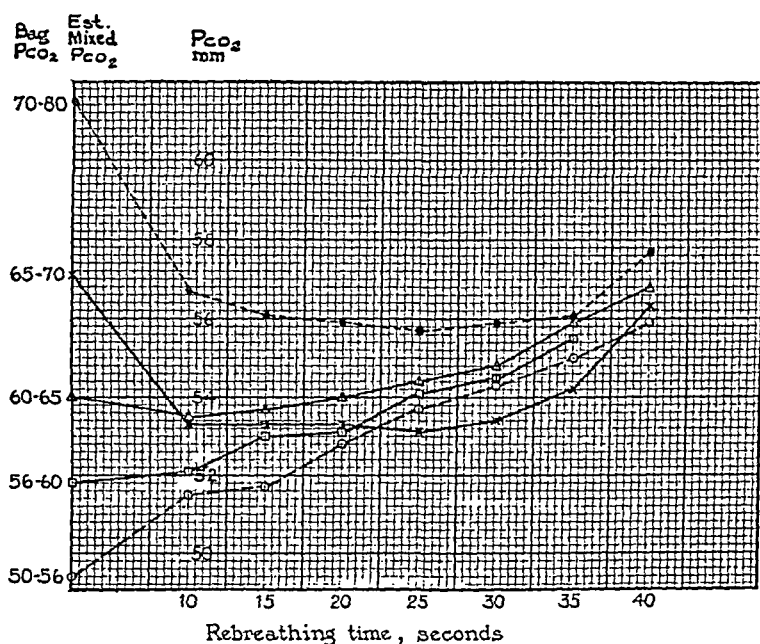


FIG. 4. OXYGENATED MIXED VENOUS CO_2 TENSIONS

Ordinates, tensions of CO_2 , mm. ($= P_{CO_2}$). Abscissae, time at which sample was taken, expressed as the number of seconds after rebreathing was begun. The "Bag P_{CO_2} " figures indicate the initial tensions in the rebreathing bag, for the particular group of experiments represented by the adjacent line and symbol. For explanation of "Estimated P_{CO_2} " tensions, see text. Each line represents the average CO_2 tension change during rebreathing, for that group of experiments.

between the fifteenth and twentieth second. This is demonstrated not only in the average figures, but in almost every one of the individual experiments: thus, of the twenty-seven experiments, there were two differences of $+0.8$ mm. CO_2 in the 15 to 20 second interval, one of $+0.6$ mm., and one of $+0.4$ mm., all the rest being between

that the arterial blood highly charged with CO_2 can dilate the tissue capillaries and venules to such an extent as to delay appreciably the recirculation of this particular part of the total blood volume? It is certainly true that the rebreathing of these higher CO_2 mixtures is associated with definite flushing of the skin and sensation of warmth.

The ten experiments performed on subject M. L. S., listed in table 5, give generally similar results to those just described. The CO_2 tension level in this subject was considerably lower than in the other, an average of 38.2 mm. for alveolar air, and of 46.7 mm. for mixed venous level. In the experiments in which the initial CO_2 tensions in the bag were 45 mm. and 48 mm., these values were too low for the establishment of an equilibrium level. In the six experiments in which the initial concentration in the bag was near 50 mm., it will be noted that the equilibrium level was in three cases attained in the 15 to 20 second interval, and in two cases in the 10 to 15 second interval. In one case the 15 second point was slightly below either of the two adjacent points. Apparently in this subject, recirculation of blood may take place in appreciable amounts in less than twenty seconds; the experiments are too few to decide this point. The two experiments with initial tensions of 60 mm. give plateau values definitely higher than the 50 mm. group. Probably this initial tension is beyond the equilibrating limit for this subject. This tension (60 mm.) is about 13 mm. higher than the apparent oxygenated mixed venous level; subject D. W. R. reached equilibrium with initial rebreathing mixtures up to 17 mm. above this level. This may have been due to the larger lung volume and residual air in the latter subject.

The definite equilibrium levels demonstrated above have, of course, been found by most workers on this subject in the past. The method which we have used, in establishing the limits of composition of re-breathed air, with which this equilibrium can be obtained, adds, we believe, further evidence in confirmation of their results.

(b) Oxygenated mixed venous tensions, method of successive rebreathings

Three experiments by this method were performed with subject D. W. R., according to the technique described above, each of these experiments being followed, after a fifteen minute interval, by another experiment of the series just described (single rebreathing

tensions only. A change in level of CO_2 curve would change the CO_2 tension also, providing pH_s values were constant.

6. When one considers the equilibrium levels in any two experiments done on the same morning or afternoon, the variations are smaller. There are five such groups, done on December 10, 14, 19, 21, and 31. In the cases of the first two of these, the subject was not at rest between experiments but got up and performed ordinary laboratory activity. The equilibrium (15 second) levels were 53.6 mm. and 52.9 mm. on December 10, and 52.8 mm. and 54.3 mm. on December 14. In the remaining three groups the subject remained at rest between experiments. The equilibrium levels were: 53.4 mm. and 52.6 mm. on December 19, 52.8 mm. and 53.4 mm. on December 21, and 54.8 mm. and 54.1 mm. on December 31. Thus it can be said that by this technique the apparent equilibrium values for successive experiments done within an hour, and with the subject at rest, differ by less than 1 mm.

7. Reference to the "Estimated mixed CO_2 tension" points in figure 4 will show that the equilibrium obtainable in the three groups II, III and IV corresponds in general with the fact that there is relatively little change necessary in the lung gases, after mixture, during the equilibration process. This is not so true of the 65 to 70 mm. group (IV) as the others, there being an excess of CO_2 in the lung air in these experiments. It is probable that lung tissue fluids are able to absorb appreciable amounts of this excess, in their change from alveolar to mixed venous tensions.

8. In the group in which the initial tensions in the bag were 70 mm. or over, there are also excellent plateaux formed, even flatter than in the preceding three groups, but their levels are definitely higher than the latter. Evidently the blood is unable to absorb all the excess of CO_2 in the lung-bag air before recirculation begins. It is to be noted that the plateau level rises only gradually above that of the lower groups, when the initial bag tension is increased from 70 to 80 mm. This has been explained in the theoretical discussion preceding.

9. There seems to be also a tendency for the CO_2 tension to rise less rapidly in the period from 25 to 35 seconds, when the initial CO_2 tensions in the rebreathing bag are high (see fig. 4). If this is true, it is difficult to explain: one would expect the opposite. Is it possible

(equilibrating both CO_2 and O_2 simultaneously), the CO_2 - O_2 relationships must be known. For blood itself, these relations can, of course, be demonstrated by constructing a nomogram. If lungs and pulmonary vein blood are in approximate equilibrium at every moment during the rebreathing procedure, then the course of equilibration, as shown by successive CO_2 and O_2 tensions, can be plotted on this nomogram. A comparison can then be made between CO_2 content as obtained by the true and by the oxygenated method.

Making provisionally this assumption of approximate lung-blood equilibrium, we constructed a CO_2 - O_2 nomogram of the blood of D. W. R., and a similar but less complete nomogram of the blood of M. L. S.

TABLE 7
CO₂ dissociation curves of reduced and oxygenated blood. Subject D. W. R.

Oxygenated		Reduced		
Total CO_2	CO_2 tension	Total CO_2	CO_2 tensions	Total O_2
<i>volumes per cent</i>	<i>mm.</i>	<i>volumes per cent</i>	<i>mm.</i>	<i>volumes per cent</i>
43.5	31.1	46.4	26.2	1.9
43.7	31.2	60.4	52.1	1.7
53.3	48.5	64.2	54.4	0.9
60.9	65.6			

To describe first the nomogram of subject D. W. R.:— CO_2 curves of reduced and of oxygenated blood were made. The technique of blood-gas analysis which we used has been described in a previous paper (33); in general it follows the method developed by Austin, Van Slyke, et al. (34). The Van Slyke-Neill (35) manometric apparatus, and the Boothby modification of the Haldane gas analysis apparatus were used for blood and gas analyses respectively. The blood was prevented from clotting by the use of small amounts of heparin, and dry NaF was added, to give a final concentration of about 0.1 per cent, as a preservative. It was thought at first that this would prevent the loss of CO_2 capacity during equilibration in the tonometers. Following a suggestion kindly made by Dr. J. H. Talbott, however, we investigated this assumption, and found it incorrect, the blood losing about 0.6 cc. of CO_2 per 100 cc. blood, per hour of equilibration at 37.5°C . We, therefore, corrected our CO_2 content figures accordingly. Three

period), for comparison. In two of these, the breath was held for 18 seconds each time, in the third (as described under Methods) the mixture was rebreathed slowly four times in the 18 seconds. This difference of technique made no significant difference in results.

A comparison of the results by the two methods is given in table 6. It will be seen that the apparent "mixed venous" level by the successive rebreathing method was considerably lower than by the other method, in all three experiments; the level in the former group being 2 mm. or more lower than that in any of the 46 experiments by the other method. The slight lowering of CO₂ values after the fourth successive rebreathing period was due presumably to a lowering of O₂ tension, the venous blood not becoming completely oxygenated. In

TABLE 6
Oxygenated mixed venous CO₂ tensions by different methods

Date	A. Single rebreathing period						B. Successive rebreathings					
	Initial bag CO ₂ tension	Seconds after beginning rebreathing					Initial bag CO ₂ tension	Rebreathing period				
		10	15	20	25	30		1st	2nd	3rd	4th	5th
		CO ₂ tension						CO ₂ tensions				
		mm.	mm.	mm.	mm.	mm.		mm.	mm.	mm.	mm.	mm.
January 28.....	61.2	53.6	53.8	54.6	54.6	55.5	50.6	50.6	49.9	50.9	50.1	49.6
February 1.....	60.2	52.6	52.4	52.7	53.6	54.0	64.7	51.9	49.4	49.4	49.3	49.2
February 4.....	57.0	51.2	52.2	52.5	53.1	53.6	58.7	52.0	50.7	50.3	50.3	49.7

the experiment of February 1, the O₂ tension in the fourth sample was 110.0 mm., in the fifth sample 89.6 mm.

It is concluded that *for this subject*, the method of successive rebreathing from the same bag without enriching the mixture with CO₂, gives equilibrium values below those obtained by the single rebreathing method.

One similar experiment with subject M. L. S. seemed to show the same thing, but these data were not enough to decide the point.

(c) *True mixed venous blood tensions*

In order that a comparison may be made between mixed venous CO₂ content of blood as derived from oxygenated tension methods, and mixed venous CO₂ content of blood as derived by true tension methods

volumes per cent (of O_2) of its proper place; this is within the experimental error of the technique employed.

It may also be noted that the CO_2 curves which were made had prac-

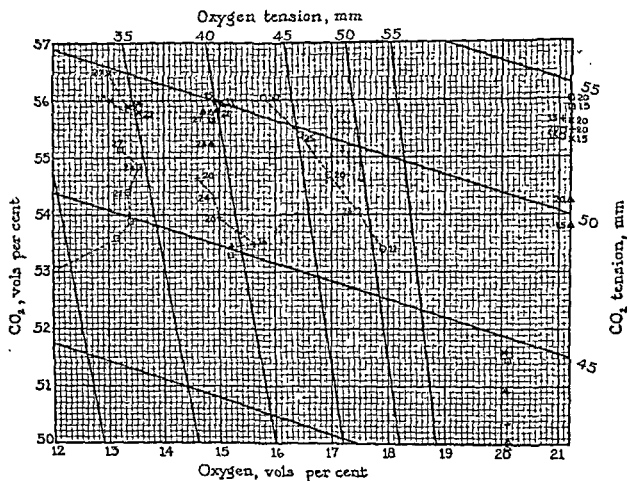


FIG. 6. CO_2 - O_2 NOMOGRAM OF BLOOD OF SUBJECT D. W. R.

Plotted on it are the progressive changes, during the course of rebreathing, in CO_2 and O_2 tensions in lung-bag air, of five experiments. The figure beside each point represents the number of seconds after beginning rebreathing, at which that particular sample was taken. At the right of the diagram, along the completely oxygenated line, are the equilibrium (15 to 20 second) tensions of a corresponding series of oxygenated mixed venous experiments. Alveolar air tensions are plotted along the 95 per cent oxygen saturation (arterial) line. Each symbol represents one day's experiments, as follows: squares, February 13; crosses, February 16; triangles, February 18; plus marks, March 7; circles, March 11.

tically the same levels as had the CO_2 curves of this subject at the time the O_2 dissociation curves were studied.

The nomograms of the blood are used in figures 6, 7, and 8.

Before considering the rebreathing experiments, it is important to give the evidence which we have for the assumption of lung-blood

points were determined on the reduced and four on the oxygenated CO_2 curves, and the curves drawn by the use of the straight line logarithmic relation of Peters, Eisenman, and Bulger (36). The data for the curves are given in table 7.

The tonometers employed for the reduced CO_2 curve determinations actually contained small amounts of O_2 . This was measured in both blood and gas phases, and a correction readily obtained by plotting the actual points on a CO_2 - O_2 diagram, and with the aid of the oxygenated CO_2 curve, extrapolating to the line of zero oxygen content. By

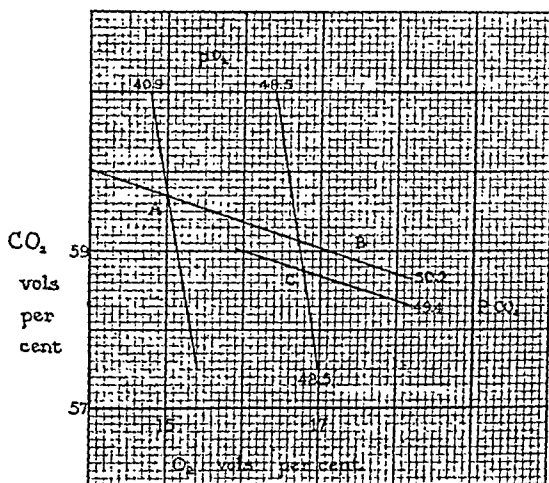


FIG. 5. EXPERIMENT OF MAY 13, 1929

Chart represents part of a CO_2 - O_2 nomogram of the blood of subject D. W. R. Point B = arterial blood at the end of 20 seconds rebreathing of mixed venous gas mixture. Point C = gas tensions in the lungs after 20 seconds' rebreathing.

the use of these extrapolated points, a completely reduced CO_2 curve was obtained.

In a previous study (33), complete oxygen dissociation curves on subject D. W. R. had been determined; these were used for the nomogram. Various workers have found that the O_2 dissociation curves of one individual change little from one year to the next (13, 37). One point was, however, determined again at an oxygen tension of 40 mm., and CO_2 tension of 50 mm.; this point, when plotted on the O_2 dissociation curve chart constructed three years previously, fell within 0.3

amount of anticoagulant, dried neutral potassium oxalate being used in this instance (final concentration about 0.2 per cent), plus enough dried NaF to give a final concentration of 0.1 per cent. The arterial blood contents of CO_2 and O_2 were measured at once, and the remainder of the blood transferred to a tonometer containing about 50 mm. CO_2 and 40 mm. O_2 . This blood was equilibrated at 37.5°C . for 50 minutes, and CO_2 and O_2 then determined on both blood and gas

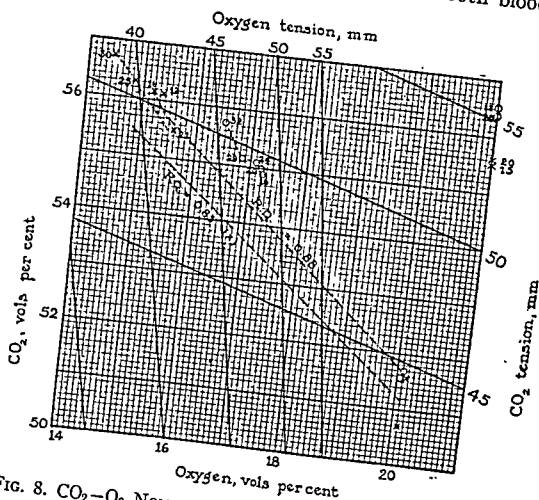


FIG. 8. CO_2 - O_2 NOMOGRAM OF BLOOD OF SUBJECT D. W. R.

For explanation see figure 6, and text. Crosses = experiments of May 3. Circles = experiments of May 13.

phases. After correction had been made for CO_2 capacity lost during equilibration, the final figures were as shown in table 8. The tensions in the lung air during rebreathing were as shown in table 9.

It is evident that from the sixteenth to the twenty-fourth second, a nearly perfect equilibrium was apparently maintained in the lung gases. Inasmuch as it probably takes several seconds for the blood to go from lung alveoli to brachial artery, the arterial blood drawn from

equilibrium during rebreathing. This experiment was performed on May 13, as follows:

The subject, after a half hour rest, applied mouthpiece and nose-clip and breathed through the apparatus (fig. 3). The two rebreathing bags had been filled with the following mixtures: Bag C, with 275 cc. CO_2 and 2,225 cc. N_2 ; Bag H with 225 cc. CO_2 , 650 cc. of air, and 1,625

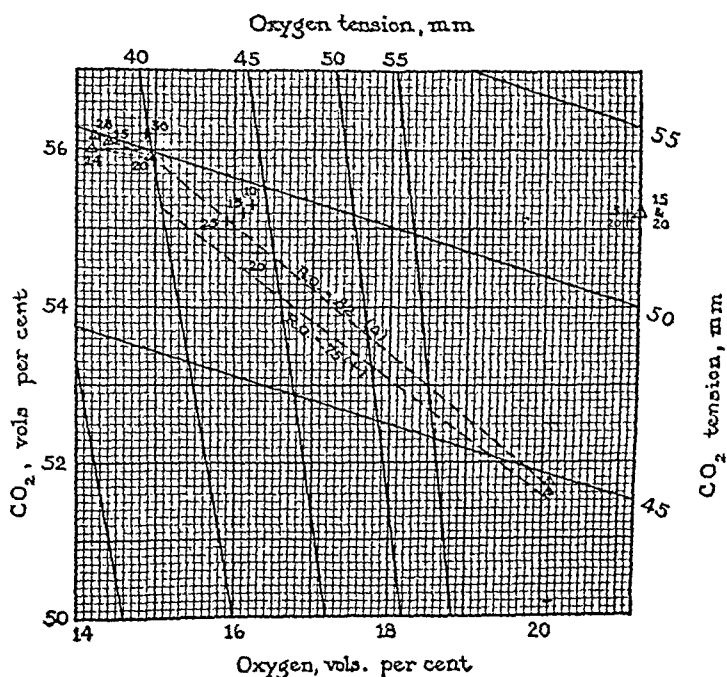


FIG. 7. CO_2 - O_2 NOMOGRAM OF BLOOD OF SUBJECT D. W. R.

For explanation see figure 6, and text. Triangles = experiments of March 15. Plus marks = experiments of March 20.

cc. N_2 . Just before the rebreathing procedure was begun, an arterial puncture was made into the brachial artery, but no blood drawn. The rebreathing was then performed, as described above. At the twentieth second of rebreathing, a signal was given and the assistant at the artery began drawing blood (under oil) and continued for the following twelve seconds. Rebreathing was continued by the subject during this interval, and the usual gas samples taken.

The blood was transferred under oil to a tube containing a small

amount of anticoagulant, dried neutral potassium oxalate being used in this instance (final concentration about 0.2 per cent), plus enough dried NaF to give a final concentration of 0.1 per cent. The arterial blood contents of CO_2 and O_2 were measured at once, and the remainder of the blood transferred to a tonometer containing about 50 mm. CO_2 and 40 mm. O_2 . This blood was equilibrated at 37.5°C . for 50 minutes, and CO_2 and O_2 then determined on both blood and gas

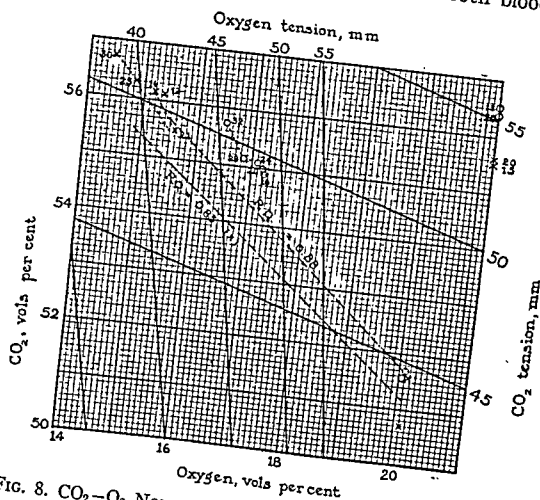


FIG. 8. CO_2 - O_2 NOMOGRAM OF BLOOD OF SUBJECT D. W. R.

For explanation see figure 6, and text. Crosses = experiments of May 3. Circles = experiments of May 13.

phases. After correction had been made for CO_2 capacity lost during equilibration, the final figures were as shown in table 8. The tensions in the lung air during rebreathing were as shown in table 9.

It is evident that from the sixteenth to the twenty-fourth second, a nearly perfect equilibrium was apparently maintained in the lung gases. Inasmuch as it probably takes several seconds for the blood to go from lung alveoli to brachial artery, the arterial blood drawn from

the twentieth to the thirty-second second must have come near being the blood which passed through the lung alveoli during the interval of equilibrium.

If equilibrium were perfect between lungs and blood, and technique of blood handling, etc., were faultless, then the arterial blood gas contents, and the lung tensions, when plotted on a $\text{CO}_2\text{--O}_2$ nomogram, should fall on the same point. Such a nomogram is given in figure 5, point *A* being that of the blood and gases in the tonometer, and the tension lines of this diagram drawn with distances and slopes

TABLE 8
Evidence of lung-blood equilibrium during rebreathing

Arterial blood contents		Blood equilibrated at 37.5°C.			
CO ₂	O ₂	CO ₂		O ₂	
volumes per cent	volumes per cent	volumes per cent	mm.	volumes per cent	mm.
59.0	17.4	59.7	50.2	15.0	40.9

TABLE 9
Tensions in lung air, same experiment as table 8

Time after starting	CO ₂	O ₂
seconds	mm.	mm.
16	49.3	48.7
20	49.4	48.5
24	49.5	48.1
29	49.6	47.0
32	50.5	45.5

corresponding to those of the more complete nomogram of this subject's blood, previously described. This is probably a justifiable extrapolation, over so small an area of the nomogram. Point *B* is the "mixed venous" blood (as drawn from brachial artery), and *C* the lung tensions after 20 seconds' rebreathing. It will be seen that the differences between the two points (*B* and *C*) are small: 0.25 volumes per cent, or 0.8 mm. of CO_2 , and 0.6 volumes per cent, or about 3 mm. of O_2 . Considering the amount of manipulation of blood necessary, this agreement is fairly good. The chief discrepancy is in oxygen, the arterial blood value being too high. The slow diffusion rate of

oxygen as compared with that of CO_2 is probably an important factor in delaying this equilibrium.

We ought to have done more experiments of this sort. It was not easy, however, to time the procedure accurately, and several unsuccessful attempts were made.

It may be concluded from this experiment that when the lung gases are at equilibrium during rebreathing (in the region of mixed venous levels), there is also approximate equilibrium between these gases and the blood leaving the pulmonary capillaries.

One cannot, of course, conclude that there is equilibrium at any moment between lungs and pulmonary vein blood, when the pulmonary artery blood varies considerably from lung tensions. However, for small lung-blood differences the discrepancy will be relatively small. One can be justified, therefore, in using blood nomograms to represent the course of equilibration during lung-blood experiments. Their direction of change will be properly represented, and if an apparent equilibrium level is reached (as indicated by constant lung tensions) then it will be known that lungs and blood have nearly the same tensions.

In the case of oxygenated mixed venous tension experiments, the demonstration of equilibrium between lungs and blood after 15 seconds' rebreathing was made, as noted above, by Field, Bock, et al. (16).

Figures 6, 7, and 8 are CO_2 - O_2 nomograms of the blood of D. W. R. on which are plotted the CO_2 and O_2 tensions of samples of rebreathed mixtures taken at successive intervals after the beginning of rebreathing using the technique described under Method (c). Each experiment is represented by a series of four to six points, connected by a dotted line, the number beside each point indicating the number of seconds after starting rebreathing, at which the sample was taken. At the extreme right, along the line of complete oxygen saturation are given, for purposes of comparison, the levels, in CO_2 tensions, of the fifteenth and twentieth second samples of the oxygenated mixed venous tension experiments. As stated above, one of these was done, in every instance, after the true mixed venous tension experiment had been completed. There may be a small error involved here, as the blood hemoglobin even with the rebreathing of high oxygen mixtures, may not become completely saturated (see Part I). Along the 95 per cent

saturation line are plotted the CO_2 tensions of alveolar air samples (corresponding to arterial blood CO_2 tensions).

It is clear that if both methods ("true" and "oxygenated") actually give at equilibrium the tensions of the incoming blood, and the latter remain unchanged during the procedures, then both equilibrium points on the nomogram will lie on the same CO_2 content line.

Each pair of experiments done on the same day (one "true" and one "oxygenated") are presented on the figures by one symbol (triangle, cross, etc.).

Perfect equilibrium on the nomogram is, of course, represented by the exact superposition of two or more successive samples.

On figure 6 are given the results of five experiments, in four of which, so far as the true mixed venous tensions were concerned, equilibrium was evidently not arrived at.

In the experiment of February 13, for example, the initial mixture in the rebreathing bag was too low both in CO_2 and O_2 . The CO_2 progressively increased during rebreathing, and the O_2 at first increased and then, after the twenty-fourth second, diminished, indicating recirculating blood. The oxygenated tension experiment reached a plateau value at 54.0 mm., as shown. This corresponds to 55.8 volumes per cent of CO_2 , well above any of the points on the other curve.

Similarly, the experiment of March 11 shows that the initial mixture was too high in O_2 and too low in CO_2 . During rebreathing the CO_2 tension progressively increased and O_2 tension diminished. The plateau level of CO_2 content in the oxygenated tension experiment was 55.4 volumes per cent. This value for CO_2 was also reached in the "true" mixed venous experiment, but only after twenty-five seconds, a time when recirculation of blood had probably begun.

The experiment of March 7 is similar to that of March 11. In the experiment of February 16, the three successive samples taken at 18, 21, and 24 seconds have the same CO_2 tensions and thus indicate an equilibrium with respect to this gas. Oxygen tensions showed an increase up to 21 seconds, followed by a decrease, suggestive evidence that the oxygen tension at 21 seconds was still below the equilibrium value. Further evidence in favor of this may be obtained by drawing an average R.Q. line (0.81) on the nomogram, starting from the alveolar air tension (as plotted on the 95 per cent O_2

saturation line). This line will be found to fall well to the right of the 18, 21 and 24 second points.

It will be noted that the 18 and 21 second CO_2 levels were higher by about 0.4 volume per cent than the plateau values in the corresponding oxygenated tension experiment. This is probably due in part at least to the failure to equilibrate with respect to oxygen.

On February 18, apparently an equilibrium was reached and maintained several seconds, as shown by the close grouping of the points. Unfortunately it is not possible to compare the equilibrium value for the CO_2 level with that of the corresponding oxygenated tension experiment, because the initial mixture used in the bag in the latter contained only 52.5 mm. CO_2 , and a plateau level was, therefore, not reached in the fifteenth to the twentieth second. The level of the true mixed venous CO_2 is, however, close to, though slightly above, the average level of equilibrium arrived at in other oxygenated tension experiments. There is a further difficulty with this experiment and that is the unusually low alveolar air tensions. These, when compared with the equilibrium point for the true mixed venous tension, give an impossibly high value for the R.Q. Where the error, or errors, occurred in this experiment we have been unable to discover.

In figures 7 and 8 are given four experiments in which a fairly close approach to equilibrium was achieved, as indicated both by the close grouping of the points, and their nearness to the R.Q. lines (slopes as determined by analyses of expired air, lines drawn from alveolar air points on figure). It will be seen that the points are clustered around the same CO_2 content lines as are the corresponding equilibrium values for the oxygenated tension experiments (plotted at the right of the nomogram). On May 13, the initial tension in the bag for the oxygenated tension experiment was over 70 mm., so that the tension at the "plateau" level, 55.3 mm., was almost certainly too high. Disregarding this experiment, the differences in CO_2 content level between the true and the oxygenated equilibrium values (average of fifteenth and twentieth second samples) were as follows: March 15, +0.7 volumes per cent (i.e., "true" higher than "oxygenated"); March 20, -0.2 volumes per cent; May 3, +0.3 volumes per cent, an average of +0.3 volumes per cent.

There seems to be a tendency for the true equilibrium values to

fall somewhat to the right of their respective R.Q. lines. The reason for this is not clear.

It will be noted in these true mixed venous experiments, that equilibrium is much more readily obtained with respect to CO_2 than O_2 . This is to be expected, and has been considered in the theoretical discussion preceding. Both the small amounts of oxygen available from the blood, and the slow diffusion rate of this gas, will delay equilibrium. We may note also that the nine experiments above described are all that were done with this technique on this subject.

We had hoped to find some evidence as to whether the concentrating effect of oxygen absorption (see Part I) would make the equilibrium CO_2 content levels of the oxygenated tension experiments higher than those of the corresponding true mixed venous tension group. The evidence, such as it is, is against this, but our data were too inexact and disperse to admit of a proper answer to this question. It is quite possible that the blood in the pulmonary capillaries after rebreathing the CO_2 — O_2 mixtures, was not more than 97 or 98 per cent saturated with oxygen, instead of 100 per cent, in which case this would tend to offset the error due to oxygen absorption.

It may, however, fairly be concluded from this group of experiments that:

1. Equilibrium of lung-bag tensions with the tensions of the incoming venous blood can be achieved with respect to CO_2 and O_2 simultaneously, only if the mixed lung gases are, at the time when rebreathing is begun, within a very few millimeters of the actual mixed venous tensions. In other words, little adjustment can be made by the absorption or liberation of gases by the blood.
2. When true mixed venous equilibrium has been attained, these values, in respect to blood CO_2 contents are at the same general level, and in each particular instance close to the corresponding value arrived at by a properly conducted oxygenated mixed venous tension determination. By "properly conducted," in the case of this subject it is meant that the initial tension of CO_2 in the rebreathing bag shall be between 56 and 70 mm.

The correspondence between the mixed venous CO_2 contents as arrived at by these different methods, coupled with the definiteness with which an apparent equilibrium can be reached by both methods,

strengthens materially, we believe, the inference that the CO_2 tensions so obtained are actually the CO_2 tensions of the mixed venous or pulmonary artery blood, at the moment when rebreathing is completed. The experiments confirm the conclusions reached in 1922 by Douglas and Haldane (13), but have extended their observations somewhat, in showing the process of equilibration more fully, and in giving more definite justification for the assumption of lung-blood equilibrium for respiratory gases.

It is of interest to calculate the volume output of the heart of this subject on the basis of our data. From analyses of expired air, on February 18, March 7, 11, 15, and May 3, the corrected figures for CO_2 output were 217.5 cc., 198.3 cc., 223.8 cc., 222.7 cc., and 204.5 cc., an average of 213.4 cc. From table 7, the average alveolar CO_2 tension is found to be 43.9 mm., and the average oxygenated mixed venous CO_2 tension of groups II, III, and IV of this table, at the end of fifteen seconds' rebreathing, is 53.3 mm. On the CO_2 dissociation curve of this subject, 43.9 mm. corresponds to 51.3 volumes per cent, and 53.3 mm. to 55.5 volumes per cent. The volume output of the heart is

thus
$$\frac{213.4}{(55.5 - 51.3) \times 10} = 5.1 \text{ liters per minute.}$$
 As this subject has a surface area of 2.00 square meters, the cardiac output per square meter is 2.6 liters. This is higher than 2.2 liters, the normal standard which Grollman (28) has worked out by the use of his acetylene method of measuring circulation rate; considering the fact that conditions in our experiments were not rigidly basal, the agreement is fairly close.

The individual figures for circulation rate, as derived from the "true" mixed venous blood experiments, show considerable variation, due chiefly to variations in alveolar air values. The mixed venous CO_2 content values for the five experiments in which an apparent equilibrium was reached were 55.9, 55.9, 55.7, 55.0, and 54.9 volumes per cent, an average of 55.5 volumes per cent,—the same as for the oxygenated experiments. The corresponding alveolar air samples (41.6, 44.4, 46.3, 44.5, and 44.7 mm.) gave an average arterial CO_2 content of 51.5

volumes per cent. The circulation rate is then
$$\frac{213.4}{(55.5 - 51.5) \times 10} = 5.3 \text{ liters per minute.}$$

In the case of subject M. L. S., two experiments were performed, each consisting of one true and one oxygenated equilibration. In order that the results might be compared, a nomogram was constructed, as described above. In this instance, however, the number of points actually determined was small: two points on the oxygenated CO_2 curve, one on the reduced curve, one point at 40 mm. CO_2 and 30 mm.

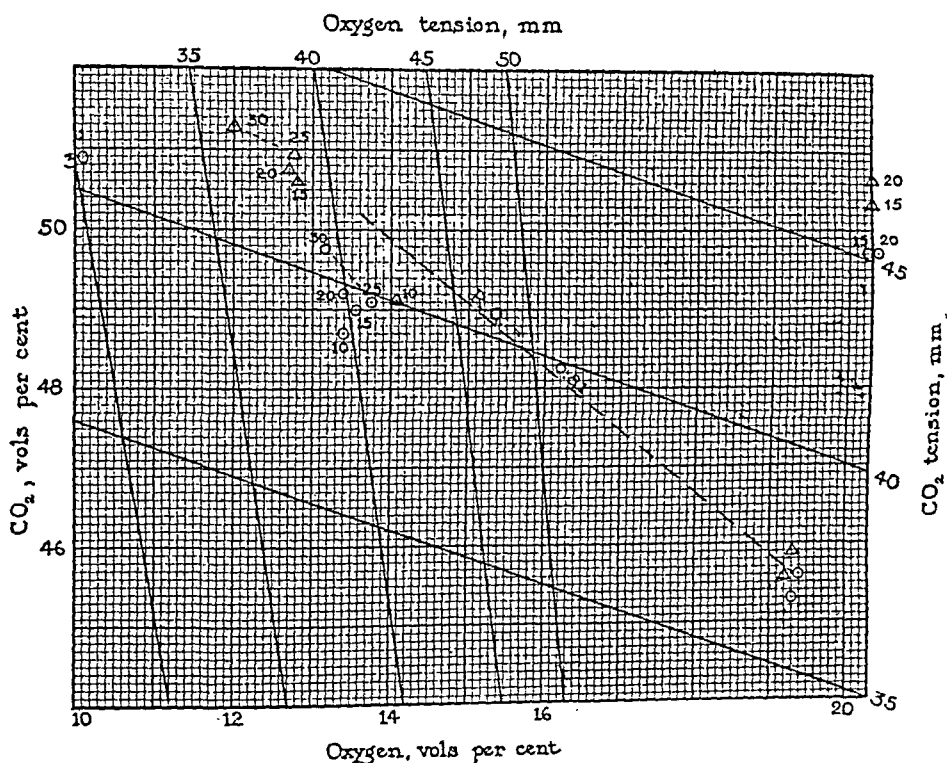


FIG. 9. CO_2 - O_2 NOMOGRAM OF BLOOD OF SUBJECT M. L. S.

For explanation see figure 6, and text. Circles = experiments of February 25. Triangles = experiments of March 1.

O_2 tensions, and one at 40 mm. CO_2 and 45 mm. O_2 tensions. These were sufficient to give the skeleton of a complete nomogram. The CO_2 tension lines were drawn by using slopes and distances similar to those of the nomogram of D. W. R.; the error involved by this procedure, over the small area under consideration, is probably not great.

The nomogram is given in figure 9, with the two pairs of experiments

plotted on it. Expired air samples were not obtained, but average R.Q. lines (≈ 0.8) have been drawn from each of the alveolar air points. It will be seen that the points in each true venous tension experiment are fairly close together. The agreement in CO_2 content level between the true and corresponding oxygenated points at equilibrium is fair: the average of fifteenth and twentieth second points show a difference of -0.6 volumes per cent ("true" less than oxygenated) in the first experiment, and $+0.25$ volumes per cent in the second. From the position of the R.Q. lines, the latter would appear to be closer to the true mixed venous point.

As in the experiments with subject D. W. R., it can be said that "true" and oxygenated methods appear to give the same general level of venous CO_2 content. The average oxygen utilization or arteriovenous difference, for this subject was 5.6 volumes per cent.

The recent experiments of Lauter with Bauman and Friedländer (25), in which blood was taken directly from the right hearts of human subjects in the resting basal state, showed the average blood flow to be about 4.5 liters per minute, and the average arteriovenous oxygen difference 5 volumes per cent. This compares well with the above results on the two subjects of our experiments, under non-basal conditions. As we studied only two subjects, no conclusion can be drawn as to the absolute accuracy of the results we have obtained, but this comparison of indirect with direct mixed venous blood determinations suggests that the unproved assumptions (see Part I) which we have made in these experiments—particularly relative to respiratory movement and recirculation of blood—do not involve any large error.

SUMMARY

From the data presented in this paper, the following summary of results obtained may be given:

1. For the investigation of mixed venous blood gases in man at rest, two normal subjects were studied. The methods employed were simple, involving the rebreathing of gas mixtures from a bag, and the taking of instantaneous samples at intervals of 5 seconds during the rebreathing period. The latter lasted usually about 30 seconds, sometimes as long as 50 seconds.

2. In the experiments in which oxygenated mixed venous blood was studied, it was found that a "plateau" level for CO_2 tensions could be obtained regularly near the fifteenth second after beginning rebreathing, providing suitable initial CO_2 tensions were used in the rebreathing bag. These tensions could be varied within well defined limits, the lower limit being a value about 2 mm. higher than the resulting equilibrium level; and the upper limit about 15 mm. above this level in the case of one subject, and 10 mm. above in the case of the other. The equilibrium level was on the average practically the same for all bag mixtures used, within these limits.

3. Below this lower limit of initial CO_2 tension, no constant plateau level was reached during rebreathing. Above the upper limit of initial CO_2 tension, plateaux were found which were definitely above those reached by the lower tensions.

4. The plateaux, or equilibrium levels of CO_2 tension, were not quite horizontal, but showed slight upward inclination. It was thought that this might be due in part to progressive oxygen absorption during the interval, and in part to recirculation of small volumes of blood.

5. The definiteness of the plateau or equilibrium levels was indicated by the facts (*a*) that they occurred regularly over the same time interval, (15 to 20 seconds), (*b*) that the deviations in slope of individual experiments from the average were much less over this interval than over earlier and later intervals, and (*c*) that widely different initial concentrations of CO_2 in the rebreathing bag were brought to the same equilibrium level at the end of rebreathing.

6. The actual equilibrium levels of mixed venous CO_2 varied with the time of day, and varied from one day to another. Successive experiments on the same morning with the subject remaining at rest, gave levels varying by less than 1 mm. of CO_2 .

7. In the experiments in which it was attempted to establish true mixed venous equilibrium (in respect to both CO_2 and O_2 tensions) between lungs and blood, it was found that the mixed lung and bag gases had to be close to the actual venous tensions, in order for equilibrium to be established in the lung gases before recirculation of blood began.

8. One experiment in which arterial blood was drawn while such a rebreathing experiment was being done, showed that, near the mixed

venous level, at the time when the lung gases were in apparent equilibrium, lung tensions and blood tensions were nearly the same.

9. By plotting on a blood nomogram oxygenated and true mixed venous tension experiments, it was found that successive experiments done by the two methods gave approximately the same venous CO_2 contents at the equilibrium levels.

10. The time when appreciable amounts of blood began to recirculate, in the cases of the two subjects of these experiments, was usually between 20 and 25 seconds. This was indicated by (a) the time when oxygenated CO_2 tensions began to show a sharp upward trend, after the plateau level; (b) the time when oxygen tensions, in the true mixed venous tension experiments, after an initial steady increase, began to decrease again.

11. Experiments in which successive rebreathing periods were employed, leaving the bag mixture unchanged between periods, gave equilibrium levels definitely lower than either of the other two groups of experiments, in the case of one of the two subjects. It was concluded that *for this subject*, the successive rebreathing method did not give mixed venous values. It was suggested also that this might account for some of the high values for circulation rate obtained with circulation rate methods in which this technique was used.

12. Calculations of circulation rate, in one of the two subjects, gave the following average values: (a) from the oxygenated mixed venous CO_2 tension values, 5.1 liters per minute; (b) from the true mixed venous CO_2 values, 5.3 liters per minute. Circulation rate per square meter of body surface (conditions not basal), 2.6 liters per minute.

13. The arteriovenous oxygen difference for one subject was 5.1 volumes per cent, for the other 5.6 volumes per cent. This compares closely with the values obtained by Lauter, by direct cardiac puncture in man.

CONCLUSION

1. By the use of a simple technique of rebreathing $\text{CO}_2\text{-O}_2\text{-N}_2$ mixtures, with samples taken every 5 seconds during the procedure, the course of equilibration of lung air with incoming venous blood has been studied. It has been found that an equilibrium can be regularly

established, for CO_2 with certain high oxygen mixtures, and for both CO_2 and O_2 with certain low oxygen mixtures. These two equilibria represent essentially the same CO_2 content levels in the (venous) blood of the subject. The mixtures of rebreathed gases that can be used to establish such equilibria can be varied within small but fairly well defined limits.

2. To accept these equilibria as representing the state of the mixed venous blood at rest involves certain assumptions; these have been described in the first part of the present study. Previous workers have shown that (a) with proper technique complete mixture of lung-bag gases is attainable; (b) "true" and "oxygenated" mixed venous CO_2 levels are the same. Our experiments have confirmed the above and have further shown that (c) the error due to progressive oxygen absorption during the oxygenated mixed venous equilibration is small, (d) lung gases and blood are in approximate equilibrium at the end of a "true" mixed venous equilibration, (e) the method of intermittent rebreathings gives false "mixed venous" equilibria in one of the subjects studied.

3. No further evidence has been obtained as to the presence or extent of error due to (a) altered respiratory movements during the procedure, or (b) recirculation within the time of the procedure, of small amounts of blood; but the comparison of our arteriovenous differences with those recently obtained by Lauter with direct cardiac puncture, suggests that the error involved here is small.

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LIVER EXTRACT, LIVER ASH AND IRON IN THE TREATMENT OF ANEMIA

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INTRODUCTION

Inasmuch as it has been demonstrated that liver and iron are potent in increasing the hemoglobin output of some patients with anemia, it seemed highly desirable to determine the effect of liver extract, liver ash, and iron in similar cases. Accordingly, we studied the response of the blood of fifty-five patients with anemia using these substances. Fourteen patients were studied following liver extract, ten following liver ash, and thirty-one following various doses of iron. In this paper we present the results of our investigations.

LITERATURE

Before proceeding it is necessary to review the information which exists regarding the value of liver extract (Number 343 Lilly) and liver ash in the treatment of the various anemias.

Besides increasing the hemoglobin and erythrocyte content of the blood in pernicious anemia (1), liver extract has been found to be effective in the treatment of the anemia of sprue (2) (3) (4) (5), tapeworm anemia (6), in some of the nutritional anemias of childhood (7) (8), in the anemia associated with intestinal strictures (9), chronic dysentery, and pregnancy (10) (11) (12) (13). When it has been given to patients with other forms of anemia the results have varied. For example, Minot, Murphy, and Stetson (13) state that one group of eleven patients showed no response to liver extract which was potent in pernicious anemia, whereas, in another group of ten patients, eight showed a slight increase in the reticulocytes, but they were not over 3.7 per cent. In the remaining two, the reticulocytes increased to from 6 to 8 per cent. From these observations they concluded that liver extract was not effective in most cases of "secondary anemia." However, they stated that if iron were added to liver extract the results were more noticeable than when either was given alone. Vaughn (14) also has reported favorable results in some forms of anemia following liver extract.

LIVER AND IRON IN ANEMIA

In the experimental anemias of dogs due to bleeding, liver extract has only approximately 10 to 20 per cent of the potency of whole liver, but when liver and liver extract are given together the hemoglobin and erythrocyte production may increase in excess of the sum of the expected separate reactions, whereas liver ash and liver extract show the sum of the two expected reactions (15). In the nutritional anemia of rats which follows a whole milk diet supplemented with iron, liver extract was potent in curing the anemia (16).

The inorganic ash of liver increases blood regenerations in the nutritional anemia of rats (16) and is about one-half as potent as whole cooked liver when fed to dogs with posthemorrhagic anemia (17). When it is given to patients with pernicious anemia, some of the preliminary phenomena of a remission, such as an increase in the reticulocytes may result, but a true remission does not occur until liver extract (Number 343) is given (18). Furthermore, it has been shown that both copper and iron salts which are found in liver ash, increase the hemoglobin output in dogs, and iron is more potent than copper (19) (20).

METHODS OF STUDY

The methods used were similar to those previously reported by us (21). All of the patients were resident in the Peiping Union Medical College Hospital during the observations. The erythrocytes, hemoglobin, and reticulocytes were determined in the usual manner. The hemoglobin of Sahli was used and checked with the oxygen capacity method so that 100 per cent was equal to 17 grams of hemoglobin or 22.7 volumes per cent oxygen. When liver extract was given, the product manufactured by Parke-Davis and Company was used. Iron was given in the form of ferrous carbonate in amounts varying from 90 to 480 mgm. of iron a day. Liver ash was exhibited in amounts which were equivalent to 300 grams of whole liver. The iron content of the liver ash used in these studies was 1.4 mgm. per gram of ash.

Results of the treatment of patients with liver extract. In studying the effect of liver extract on the course of various anemias, we selected patients with the types of anemia which, in our experience, usually responded to whole liver feeding. We adopted this procedure in order to determine whether liver extract was beneficial in such cases, and we felt that there was nothing to be gained by studying effects of liver extract in anemias which did not respond to whole liver. This group included anemias of childhood associated with nutritional disturbances, anemias of pregnancy, hookworm infestation, anemias associated with chronic dysentery, and posthemorrhagic anemia. In some cases liver extract was given alone. It usually was given for two weeks or longer, and in those who received iron, it was added after

the maximum effect had been obtained from liver extract. That is to say, iron was added after the hemoglobin had increased on liver extract and remained at a stationary level for several days. The hemoglobin and erythrocyte values recorded in the table represent the values of the hemoglobin before and after liver extract treatment, and not the number of red cells and amount of hemoglobin after complete recovery. The results are summarized in table 1. The erythrocytes varied from 600,000 to 4,000,000 per cubic millimeter,

TABLE 1
Results of treatment with liver extract

Case	Type of anemia	Red blood cells		Hemoglobin		Reticulocytes	
		Before treatment	After treatment	Before treatment with liver extract	After treatment with liver extract	Before treatment	After treatment
		millions per cu. mm.	millions per cu. mm.	per cent	per cent	per cent	per cent
1	Childhood	0.80	3.50				
2	Dysentery	0.60	2.50	18	60	0	35
3	Dysentery	1.00	1.60	18	55	1.2	38
4	Childhood	3.00	5.25	15	20	0	5
5	Hookworm	3.50	4.50	50	75	0	6
6	Pregnancy	2.00	3.50	45	70	4	10
7	Dysentery	4.00	4.50	45	62	2	11
8	Dysentery	2.25	2.75	40	50	0	3
9	Dysentery	2.75	3.50	55	65	1	7
10	Dysentery	3.05	4.90	40	50	0	4
11	Dysentery	1.35	1.25	52	100	0	6.4
12	Dysentery	1.15	1.50	35	40	1.6	1
13	Posthemorrhagic	1.75	2.20	14	20	0	3
14	Tuberculosis of intestine	2.00	2.00	32	35	2	5
				45	40	4	10

and the hemoglobin from 18 to 55 per cent. In ten of the fourteen patients studied there was a definite acceleration of the hemoglobin regeneration. In some it was considerable, while in the others it was not very striking. In the remaining patients, there were no signs of improvement. In some of those showing improvement the effect of liver extract was enhanced by iron. Chart 1 shows the results of liver extract in Case 1.

Chart 2, Case 2, shows the response of the blood following liver extract in a patient with anemia associated with chronic dysentery.

LIVER AND IRON IN ANEMIA

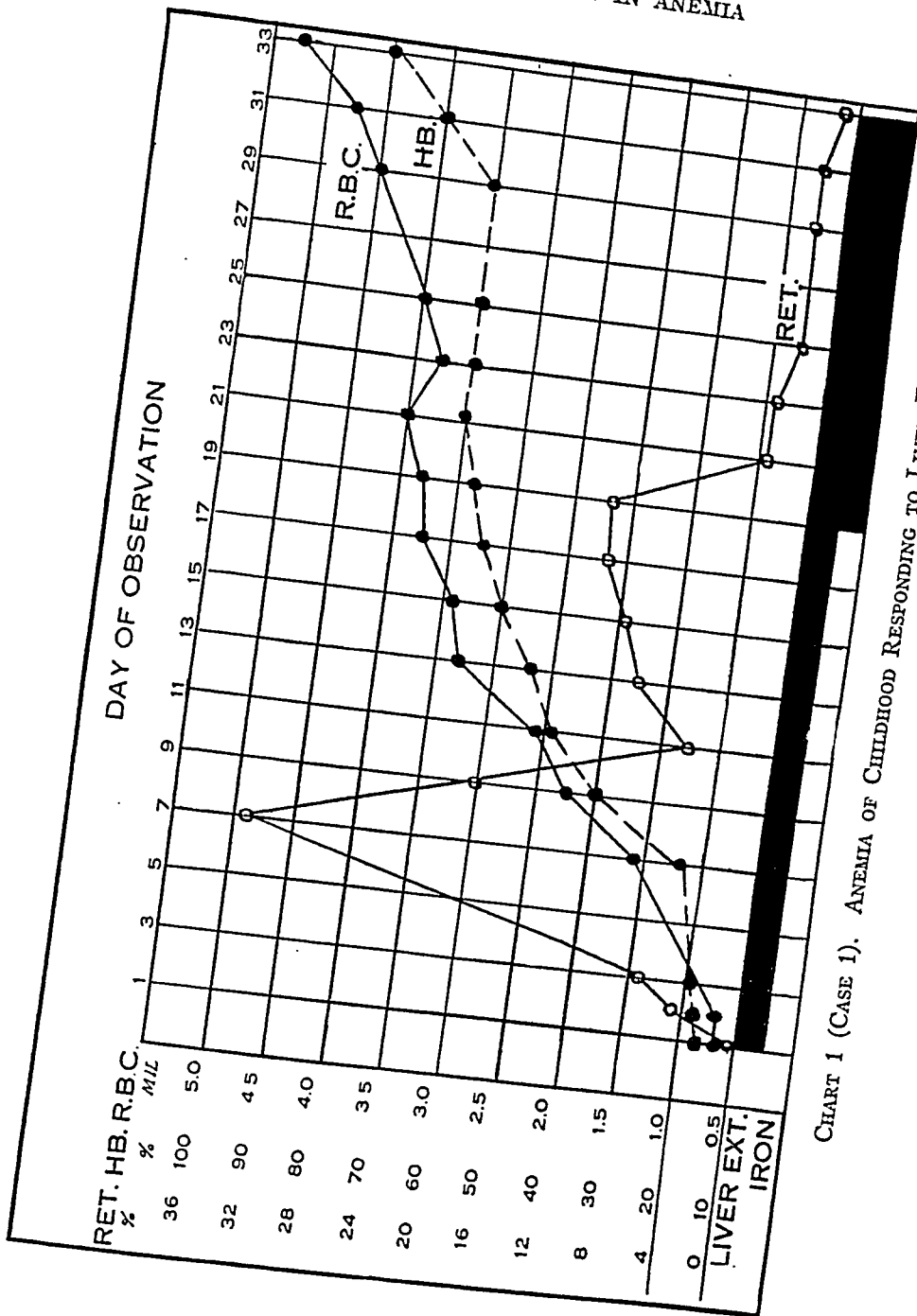


CHART 1 (CASE 1). ANEMIA OF CHILDHOOD RESPONDING TO LIVER EXTRACT

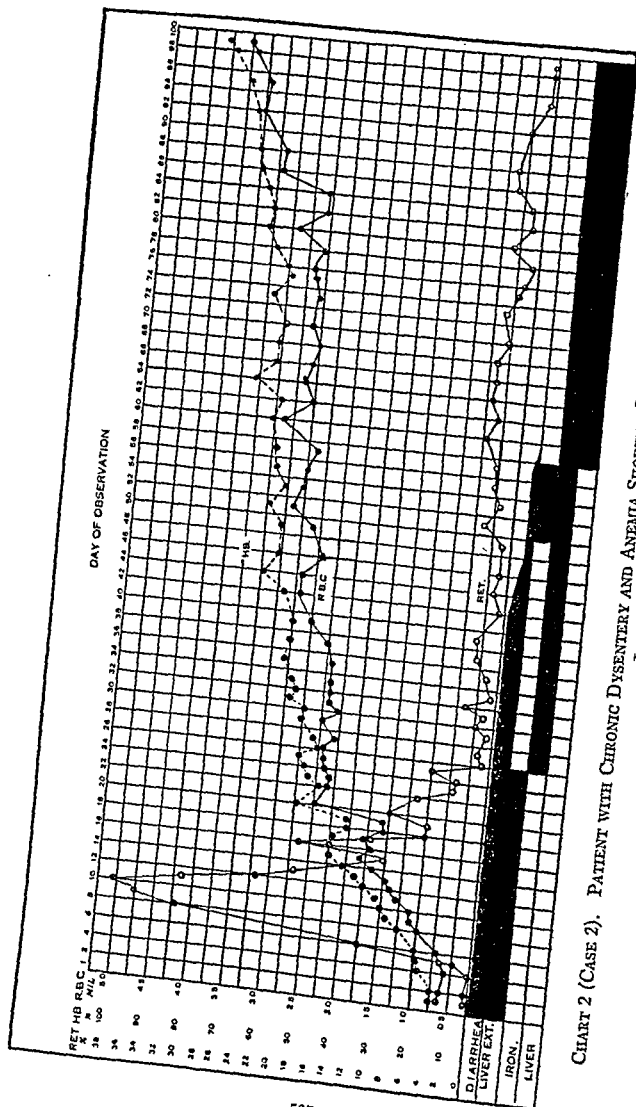


CHART 2 (CASE 2). PATIENT WITH CHRONIC DYSENTERY AND ANEMIA SHOWING MARKED IMPROVEMENT FOLLOWING LIVER EXTRACT

LIVER AND IRON IN ANEMIA

Chart 3, Case 3, illustrates the response of the blood following iron after liver extract failed to produce improvement.

In the patients who responded favorably to liver extract there was an increase in number of reticulocytes in the peripheral blood, the number at the peak of the rise depending upon the level of erythrocytes before treatment was begun.

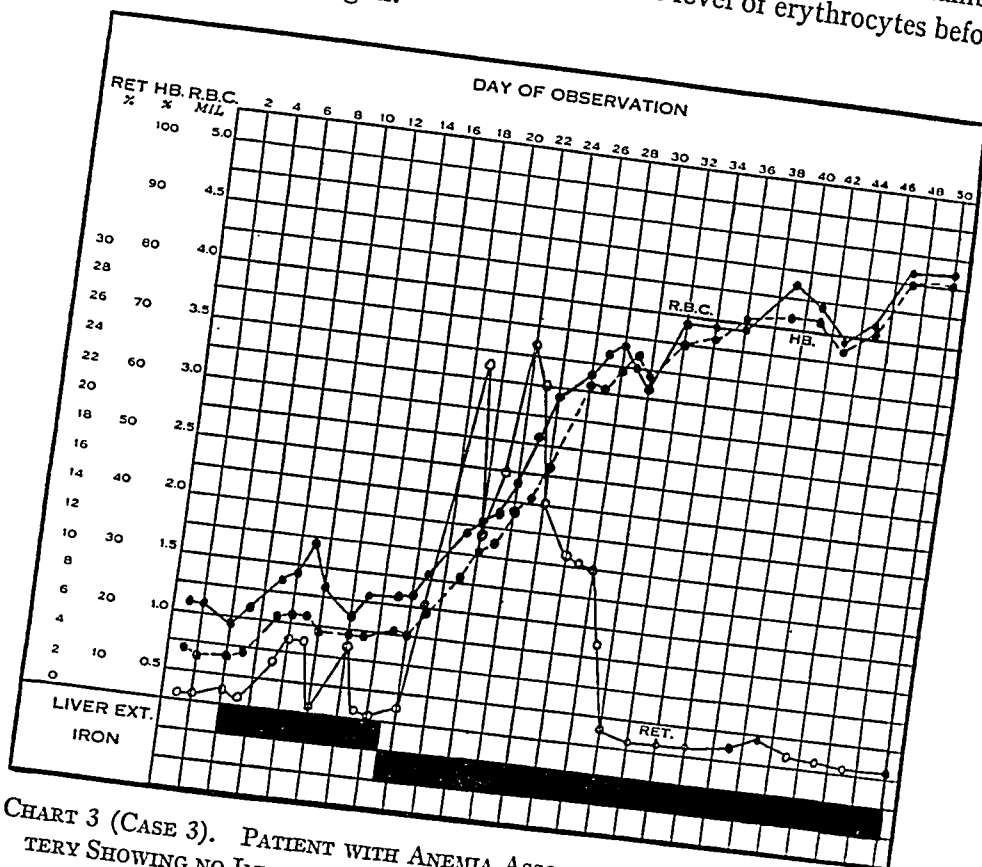


CHART 3 (CASE 3). PATIENT WITH ANEMIA ASSOCIATED WITH CHRONIC DYSEN-
TERY SHOWING NO IMPROVEMENT FOLLOWING LIVER EXTRACT BUT A PROMPT
RECOVERY FOLLOWING IRON

The value of liver ash in the treatment of anemia when compared with iron. In a previous paper we (21) pointed out that iron was of value in the treatment of some forms of anemia and the daily rate of hemoglobin regeneration following iron in the cases reported varied between 0.5 and 2.2 per cent a day. It was also noticeable that the effect of liver was enhanced by iron feeding. As a result, we proceeded to

determine whether the response to liver was due to its inorganic constituents, of which iron is one, or to other substances. We studied the course of the blood in patients with anemia and the value of liver ash as compared with iron and liver. The liver ash was prepared by mincing the liver in a meat grinder and drying it in an oven until a fine brown powder was obtained. It was then burned with a hot flame until a carboniferous mass was formed. This was then ground in a mortar and heated again until a fine gray powder was obtained. The results of liver ash feeding are summarized in table 2. The values recorded correspond to those at the beginning and end of treatment.

TABLE 2
Results of the treatment of anemia with liver ash

Case	Red blood cells		Hemoglobin		Reticulocytes		Type of anemia
	Before liver ash	After liver ash	Before liver ash	After liver ash	Before treatment	After treatment	
	millions	millions	per cent	per cent	per cent	per cent	
15	3.75	4.0	42	50	0	4.8	Dysentery
16	3.50	4.0	40	45	0.4	1.8	Nutritional
17	3.00	4.25	52	65	0	4	Edema disease
18	2.11	2.44	37	38	0.1	1.2	Hookworm
19	4.00	4.0	70	75	4	0	Edema disease
20	2.57	2.50	34	33	1	1.0	Posthemorrhagic
21	2.02	1.92	43	35	0.4	3	Cirrhosis of liver
22	3.46	4.41	43	55	0.8	3.4	Posthemorrhagic
23	3.33	3.28	53	47	1.6	2.6	Dysentery
24	2.86	2.72	65	58	2.6	4.8	Dysentery

In several there was a slight increase of the erythrocytes, hemoglobin, and reticulocytes, but complete recovery was never observed.

Following the observations of liver ash, liver or iron was given and the results compared. In most instances, the changes were striking and may be illustrated clearly by means of charts.

Charts 4 and 5 illustrate the course of the blood of two patients with a chlorotic type of anemia associated with malnutrition. In one (chart 4, Case 15) no improvement followed liver ash. When the equivalent amount of whole liver was given daily for fourteen days, there was slight improvement with an increase of the various elements of the blood; but following large doses of iron, recovery occurred

LIVER AND IRON IN ANEMIA

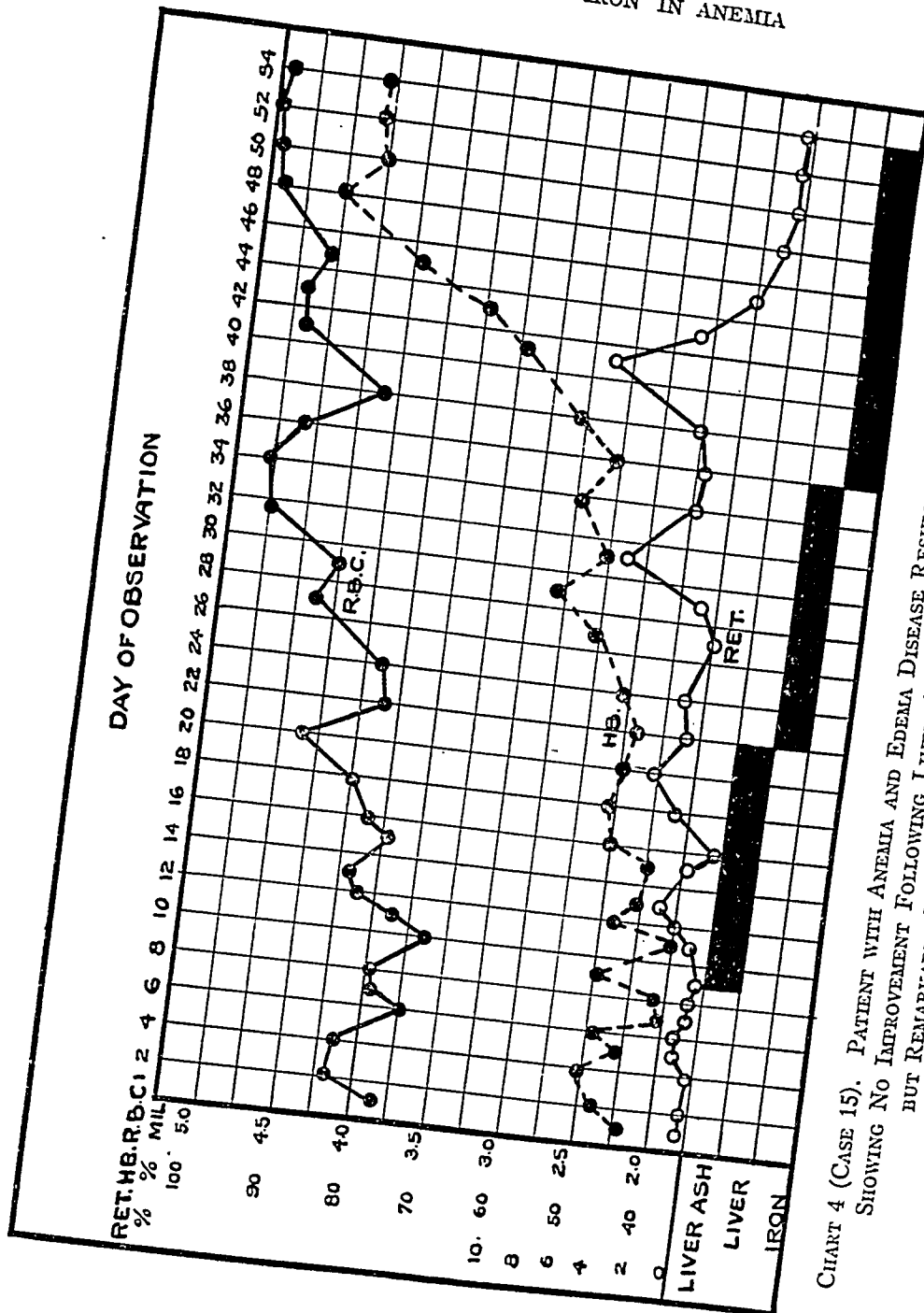


CHART 4 (CASE 15). PATIENT WITH ANEMIA AND EDEMA DISEASE RESULTING FROM AN INADEQUATE DIET
SHOWING NO IMPROVEMENT FOLLOWING LIVER ASH, BUT REMARKABLE RECOVERY FOLLOWING 0.48 GRAM OF IRON A DAY

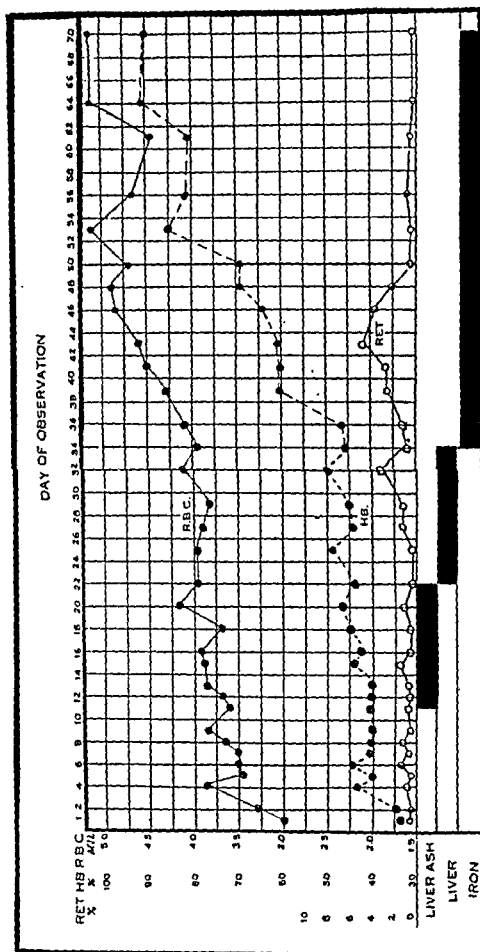


CHART 5 (CASE 16). PATIENT WITH ANEMIA DUE TO FAULTY DIET SHOWING NO IMPROVEMENT FOLLOWING LIVER ASH OR LIVER, BUT RECOVERING FOLLOWING 0.48 GRAM OF IRON A DAY

promptly. In the other patient (chart 5, Case 16) no demonstrable improvement resulted from liver ash or whole liver, whereas complete recovery followed iron. These two cases illustrate that the inorganic elements of liver were of little value in these patients when given in amounts equivalent to 300 grams of whole liver, whereas large doses of iron were effective.

Charts 6 and 7 illustrate the difference between the reactions of liver ash and iron. In Case 17 (chart 6) there was temporary improvement of the anemia following hospital diet and the subsidence of the symptoms of dysentery. This was followed by an exacerbation of the dysentery and a fall in hemoglobin and red blood cell count. Following liver ash there was an increase of red cells, and a slight increase in the hemoglobin content of the blood. However, the hemoglobin did not return to normal until after the administration of iron.

In Case 18 (chart 7) the anemia was associated with hookworm infestation. There was no response to liver ash, but marked improvement following iron. Whole liver caused no further improvement.

In Case 19 (chart 8) the anemia was associated with undernutrition and edema disease. There was a satisfactory response to iron which was not enhanced by liver ash.

From the above cases it is clear that liver ash was of little demonstrable value when given in amounts which were equivalent to 300 grams of whole liver a day. In some a similar amount of whole cooked liver was more effective than liver ash. However, when these results were compared with iron, the contrast was striking and requires further comment.

On the basis that iron is one of the potent factors of liver ash, it is surprising that we did not observe more conspicuous results following its exhibition and more particularly since the response in the same patients was so remarkable following large doses of iron.

In attempting to explain why this should be so, it is necessary to keep in mind the available facts regarding the production of hemoglobin following iron feeding in animals. The question continues to be a controversial one. In the experience of different investigators, the results of iron feeding have not been in agreement. These differences are, no doubt, due to the fact that iron has been tested in the various anemias of rats, and the results compared with those following

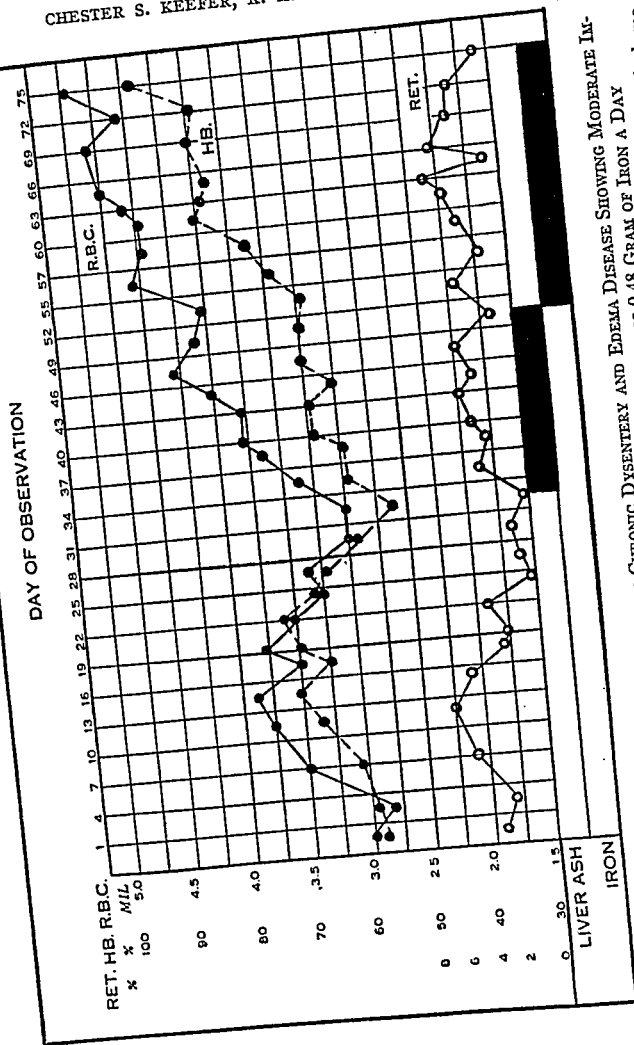


CHART 6 (CASE 17). ANEMIA ASSOCIATED WITH CHRONIC DYSENTERY AND EDEMA DISEASE SHOWING MODERATE IMPROVEMENT FOLLOWING LIVER ASH AND COMPLETE RECOVERY FOLLOWING 0.48 GRAM OF IRON A DAY

The temporary improvement at the beginning of the observations was followed by a period of depression of the hemoglobin and red cells due to an exacerbation of the dysentery.

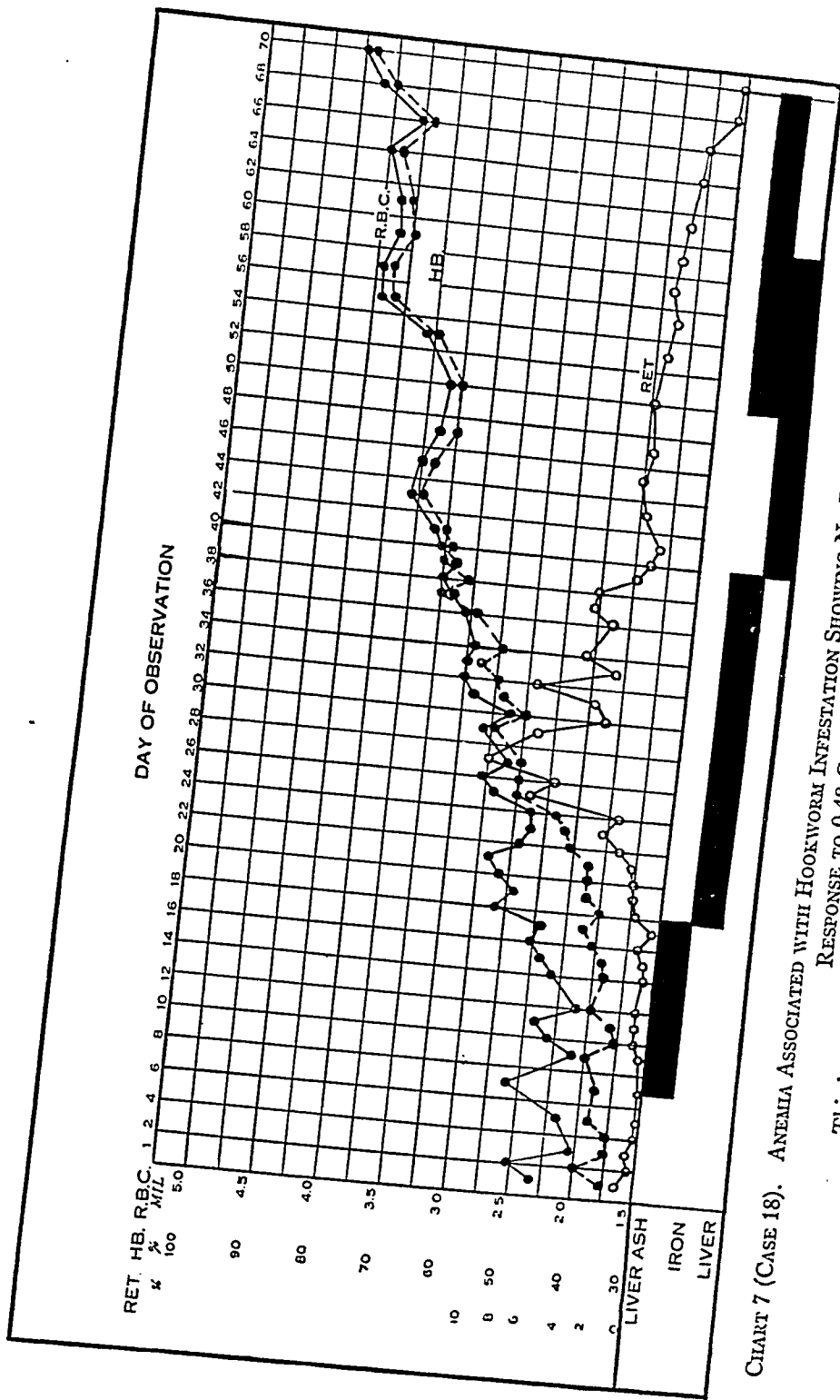


CHART 7 (CASE 18). ANEMIA ASSOCIATED WITH HOOKWORM INFESTATION SHOWING NO RESPONSE TO LIVER ASH, EXCELLENT RESPONSE TO 0.48 GRAM OF IRON A DAY

This improvement occurred while the patient continued to carry the worms

DAY OF OBSERVATION

RET. HB. RBC.
% MIL
% 100 5.0

90 4.5

80 4.0

70 3.5

60 3.0

50 2.5

40 2.0

30 1.5

20 1.0

10 0.5

0 0.0

IRON

LIVER ASH

ANEMIA ASSOCIATED WITH UNDERNUTRITION AND EDEMA DISEASE RESPONDING TO 0.18 GRAM OF IRON A DAY

CHART 8 (CASE 19).

The effect of iron was not enhanced by liver ash

RET. HB. RBC. HB. RET.

1 19 22 25 28 31 34 37 40 43 46 49 52 55 57

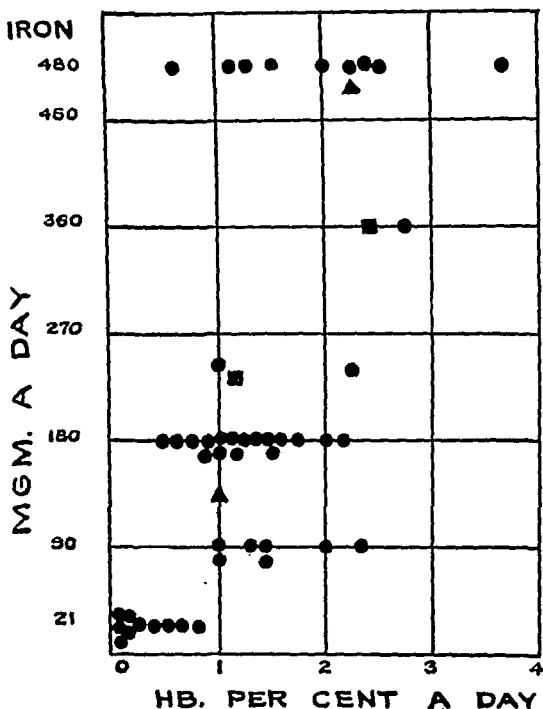
iron feeding in other animals with anemias of different cause. Since there are several factors involved in hemoglobin production, and the iron effect is only one, it is not surprising that the results have varied. This question was reviewed recently by Robscheit-Robbins (22). She emphasized these differences quite clearly and concluded from her own observations on dogs with anemia due to bleeding, that large doses of iron were more effective than small ones. Whipple and Robscheit-Robbins (23) found that the maximum effect could be obtained following 40 mgm. of iron a day. When more iron was given the output of hemoglobin was not increased, and since this amount of iron was always in excess of that which was contained in the hemoglobin produced, it was concluded that the action of iron was catalytic in nature and caused a readjustment of the internal protein metabolism so that more hemoglobin was produced.

It is evident from these studies that the dosage of iron is of considerable importance in promoting hemoglobin regeneration, whereas the exact mechanism by which it produces its effect is not quite clear.

In considering the part played by iron in the production of hemoglobin in man, it will be acknowledged generally that it is of value in the treatment of some forms of anemia. It has been emphasized by Mettier and Minot (24) that large doses of iron are necessary in order to obtain the maximum effect. To gather further information regarding this question we have made observations in thirty-eight patients who received various amounts of iron daily.

The iron was given in the form of ferrous carbonate, so that the amount of iron varied between 90 and 480 mgm. daily. The results are plotted in chart 9. Each dot represents a patient, and the position of the dot on the chart illustrates the rate of hemoglobin regeneration following the dose of iron exhibited. The two squares and two triangles represent the rate of hemoglobin regeneration in two patients following different doses of iron. In these patients, the large dose of iron increased the hemoglobin regeneration more than the smaller one. However, from the chart it is clear that in some cases at least, a positive effect was produced by as little as 90 mgm. of iron a day. When one compares the hemoglobin regeneration following 21 mgm. of iron daily, when given in the form of liver ash, with that following larger amounts of iron, the results are striking. The dots in the lower

left-hand corner of the chart represent the hemoglobin regeneration following liver ash. Following these doses the hemoglobin output was slight, whereas, following larger doses of iron the increase was consider-



ably greater. The results of a study of seven patients who just received liver ash and then iron are recorded in table 3.

Besides calculating the average daily increases in hemoglobin following various doses of iron, we studied the response of the reticulocytes in order to determine whether large doses of iron were more effective in increasing the reticulocytes in the blood than small doses. These data are plotted in chart 10. The percentage of reticulocytes following various amounts of iron is charted with the total number of erythrocytes before treatment. The squares represent the response following 480 mgm. of iron; the dots, 180 mgm.; the triangles, 90 mgm.; and the diamonds, 21 mgm. in the form of liver ash. It is readily seen

TABLE 3
Results of the treatment of anemia with varying doses of iron

Case	Before liver ash		After liver ash Iron content 0.021 gram		Before iron		After iron		Daily dose of iron	Reticulocytes	
	Red blood cells	Hemo- globin	Red blood cells	Hemo- globin	Red blood cells	Hemo- globin	Red blood cells	Hemo- globin		Follow- ing liver ash	Follow- ing iron
	millions	per cent	millions	per cent	millions	per cent	millions	per cent		per cent	per cent
15	3.75	42	4.0	50	4.0	50	5.25	90	0.48	4.2	9
16	3.50	40	4.0	45	4.0	45	5.25	90	0.48	1.8	5
17	3.0	52	4.25	65	4.25	65	5.0	90	0.36	1.0	7
18	2.11	37	2.44	38	2.44	38	3.5	70	0.48	1.2	12
20	2.57	34	2.50	33	2.50	33	5.0	90	0.36	1.0	7
21	2.02	43	1.92	35	1.92	35	4.5	88	0.18	3.4	9
23	3.33	52	3.28	47	3.28	47	4.5	85	0.48	2.6	3.4

that as a general rule the lower the red blood cell count at the beginning of treatment, the greater was the increase in reticulocytes. However, this was only true of patients in whom the dose of iron was large enough to produce a positive effect. For example, the response following iron given in doses of 21 mgm. (diamonds) was never very conspicuous, but when larger doses were given to the same patients, an increase in the reticulocytes was evident. (See table 3.)

From these observations it would seem justifiable to conclude that when anemias respond to iron therapy the dosage is of importance, and large doses are relatively more effective than small ones. This has been emphasized previously by Whipple and Robscheit-Robbins

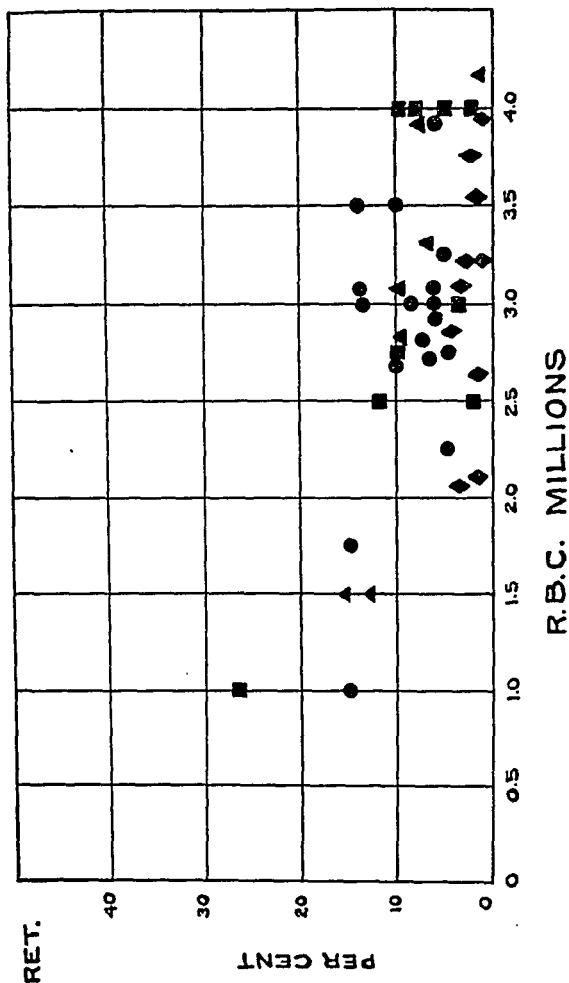


CHART 10. THE RESPONSE OF THE RETICULOCYTES FOLLOWING VARIOUS DOSES OF IRON

The percentage of reticulocytes at the peak of the rise is charted according to the number of red cells present before treatment was begun. The diamonds represent the response following 21 mgm. of iron a day when given in the form of liver ash. The triangles, dots and squares represent 0.09 gram, 0.18 gram and 0.48 gram of iron respectively.

and Mettier and Minot. It would appear, however, that an increase in hemoglobin regeneration may be observed in some cases following 90 mgm. of iron daily. In others larger doses seem necessary.

It remains to discuss why iron is effective in some of the human anemias and not in others. We have pointed out the necessity of adequate amounts of iron and Mettier and Minot (24) have suggested that the reaction of the gastric secretion may be of considerable importance in determining its effectiveness. In other words, it appears that the dosage must be adequate and the conditions for its absorption favorable. There is another possibility which must be considered, namely, the value of copper in increasing the hemoglobin content of rats with anemia resulting from a milk diet supplemented with iron. Its value, however, as a supplement to iron in the various human anemias, remains unclear. Mills (25) has reported favorable results in some patients with a hypochromic anemia following iron and copper, and believes that copper is necessary for the maximum iron effect in this form of anemia. Moreover, he attributes the striking results obtained in some of these anemias following liver and iron to the copper content of the liver. Whether copper is necessary for hemoglobin production in other forms of anemia requires further investigation.

It would appear from these observations that the iron effect is only one factor necessary for hemoglobin regeneration, and this had been designated as the *salt effect* by Whipple and Robscheit-Robbins (23). In some cases, when iron is lacking, recovery will not take place until the iron is provided; in other instances, when it is available and other substances necessary for hemoglobin regeneration are lacking, recovery will not occur until they have been supplied. The exact nature of these various factors require further study.

Response of reticulocytes following various forms of treatment. When a patient with pernicious anemia recovers following liver or liver extract there is an increase in the reticulocytes; the number appearing in the circulatory blood depends upon the amount of liver or liver extract given and the number of erythrocytes present at the beginning of treatment (26). That is to say, the lower the erythrocyte count before treatment is begun and the larger the amount of liver or liver extract given, the greater the number of reticulocytes in the peripheral blood at the peak of the rise.

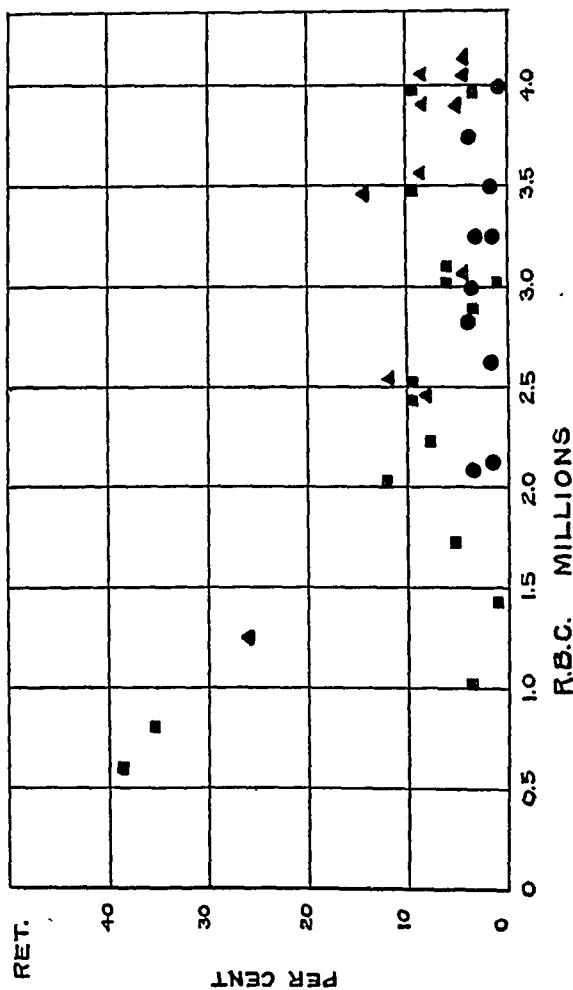


CHART 11. THE RESPONSE OF THE RETICULOCYTES IN PATIENTS WITH VARIOUS FORMS OF ANEMIA FOLLOWING DIFFERENT KINDS OF TREATMENT

The squares represent the response to liver extract, the triangles, iron, and the dots, liver ash. The reticulocytes at the peak of the rise are plotted against the red cell level before treatment was begun.

We (27) found in a study of the response of the reticulocytes following the treatment of various forms of "secondary" anemia, that the number of reticulocytes appearing in the blood depended upon the severity and cause of the anemia and the kind of treatment. In studying the response of the reticulocytes in these patients we have made chart 11. The triangles represent the reticulocyte response to iron; the squares, the response to liver extract; and the dots, that following liver ash. The most conspicuous results followed liver extract and iron, whereas, liver ash produced only slight changes. However, one may say that whenever a patient responded to either liver extract or iron, the lower the red blood cell count, the higher were the reticulocytes at the peak of the rise. The reason for the slight response of the reticulocytes following liver ash was probably due to the small amount of iron which was present.

SUMMARY AND CONCLUSIONS

We have reported the results of a study of the effect of feeding liver extract, liver ash, and iron to patients with various forms of anemia and the following facts were evident.

1. Liver extract was of demonstrable value in increasing the regeneration of hemoglobin in some of the obscure nutritional anemias of childhood, in some of the anemias associated with dysentery, hookworm infestation, and pregnancy. In some cases its effect could be enhanced by iron.
2. Liver ash, when given in amounts which were equivalent to 300 grams of whole liver, was of little demonstrable value in increasing hemoglobin regeneration. In some cases there was a slight increase in the reticulocytes, hemoglobin, and erythrocytes, but the results were never as conspicuous as those obtained with liver extract or large doses of iron.
3. Iron was effective in the treatment of various forms of anemia. Large doses were more effective than small ones. In many instances the effect of iron exceeded both liver, liver extract, or liver ash, and in some cases a favorable response did not occur until iron was added.
4. When recovery from the anemias followed either liver extract or iron there was an increase in the reticulocytes of the circulating blood. The lower the erythrocyte count before treatment, the higher were the reticulocytes at the peak of the rise.

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THE RELATIONSHIP OF BLOOD URIC ACID CONTENT TO THE STATE OF RENAL FUNCTION IN NEPHRITIS.

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ERRATA

VOL. VIII, No. 4, JUNE, 1930, PP. 506-507

Article by Shohl, Goldblatt, and Brown, "The Pathological Effects upon Rats of Excess Irradiated Ergosterol"

Page 506, footnote 2, add: A test of this preparation by Miss D. N. Smith, showed that 0.0001 to 0.00025 mgm. is sufficient, when fed to rats on the Steenbock diet, to protect against rickets.

Page 507, footnote 3: Delete second sentence.

VOL. IX, No. 3, DECEMBER, 1930, P. 381

Article by Paul Reznikoff, "Nucleotide Therapy in Agranulocytosis"

Page 381, footnote to be appended: Dr. Henry Jackson, Jr. suggests to me that the compounds adenine sulfate and guanine hydrochloride should not be called nucleotides but purine salts derived from nucleotides. The work of Doan quoted in this article was carried out with nucleotides containing these purines.

determination as an index of renal function by reports of its increase in many unrelated conditions. Gout, leukemia, and pneumonia had for many years been known to cause this increase, but newer work indicated that in addition, a rise in the level of blood uric acid could be found in pernicious anemia (7), carbon monoxide poisoning (8), toxemias of pregnancy (9), erythemia (10), eczema (11), and during

BLOOD URIC ACID IN NEPHRITIS

the first few days of life (12). Umeda (13) had shown that normal individuals excreted only small amounts of uric acid when on excessively high fat diets, but it remained for Lennox (14, 15) and Harding (16) and his collaborators to show that this decreased excretion was accompanied by a remarkable increase in the blood uric acid either during starvation, or as the result of a ketogenic diet.

Folin, Berglund and Derick (17), injected uric acid into normal men and found that the excretion period following injection varied be-

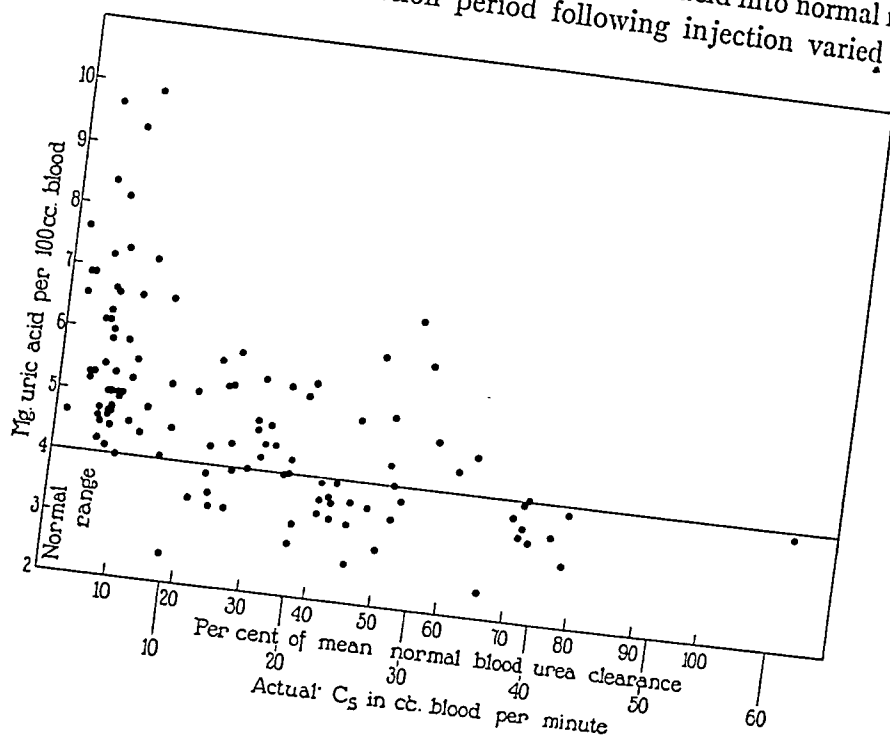


FIG. 1

tween one and four days. They were able to recover from 30 to 90 per cent of the amount injected, the remainder being apparently destroyed.

Although the estimation of blood uric acid as an aid to estimating the state of renal function in nephritis has apparently been abandoned in many clinics, its use has been so general that it still appears desirable to report a comparison of blood uric acid contents with determinations of the blood urea clearance, which has shown itself to be especially reliable and sensitive as a measure of renal function⁽¹⁾.

The urea nitrogen of blood and urine was determined in our cases by the Van Slyke-Cullen urease aeration method (18) (the gasometric urease methods had not yet been introduced). The uric acid was determined in whole blood filtrate by the method of Benedict (19).

Figure 1 presents the data obtained from 117 tests on 30 patients. Twenty-one of these patients had chronic hemorrhagic Bright's disease, and the remainder were distributed as follows: acute hemorrhagic, 2; chronic degenerative, 3; arteriosclerotic, 3; cardiac failure, 1. The approximate upper limit of normal, taken as 4 mgm. of uric acid per 100 cc. of blood, is indicated by the horizontal line drawn at that level across the chart.

The data in figure 1 indicate the following:

1. Normal blood uric acid (2 to 4 mgm. per cent) occurs in a large proportion of nephritic cases with urea excreting power from normal down to 20 per cent of normal.

2. Moderately increased blood uric acid (between 4 and 6 mgm. per cent) may accompany either slight renal damage or the most extreme loss of function.

3. Blood uric acid over 7 mgm. per cent appears to be caused by nephritis only when the latter has reached an advanced stage with urea excreting function less than one-fifth normal. Since similar uric acid values may be caused by other pathological conditions, however, even such high values may not always be accepted as evidence of renal damage.

The irregular correlation of blood uric acid to renal function is what might be anticipated from the fact that uric acid is removed from the human body only partly by excretion, part being destroyed.

Of the two exceptional cases, in which the uric acid rose above 6 mgm. per cent while the clearance was approximately 50 per cent of normal, severe cardiac decompensation was present in one and acute streptococcic pharyngitis in the other. In this connection it may be said that the records of all patients with urea clearances above 20 per cent of normal, together with abnormally high values for blood uric acid, were carefully examined. They were all receiving diets with 40 grams or more of protein daily, adequate in both carbohydrates and total calories, so that starvation acidosis could not have been present as a complicating factor.

SUMMARY AND CONCLUSIONS

The concentration of uric acid in the blood of 30 patients with renal disease has been compared with the standard blood urea clearance determined simultaneously.

The determination of blood uric acid as an indicator of renal function appears to be of little value. Normal blood uric acid may occur despite 80 per cent loss of urea excreting power. When rise of blood uric acid does occur there is, except in some terminal cases, little correlation between the extent of rise in blood uric acid and the extent of fall in renal function.

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PHYSIOLOGICAL STUDIES OF FAINTNESS AND SYNCOPE

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(Received for publication September 17, 1930.)

During experiments to determine the relationship of the ethyl iodide content of alveolar air and arterial blood in the upright position a number of subjects experienced faintness and two collapsed. Such an incident, occurring during a time when measurements of respiration and circulation were in progress, provided unusual opportunity for complete observation of the phenomena of faintness and syncope. Our study of the six cases which form the subject of this report represents an effort to improve such opportunities. Our attempts to enlarge the series met with failure. It seemed to us that, as soon as the danger of fainting became known to the subjects, syncope no longer occurred. Those who collapsed were taken by surprise.

Studies of fainting attacks in soldiers with irritable hearts have been made by Cotton and Lewis (1). A case of syncope following short periods of cardiac arrest has been carefully studied by Laslett (2). In both of these vagal stimulation was identified as the chief factor; in our cases this appears to have been of secondary importance only.

METHODS

The apparatus and methods used for the determination of cardiac output (3) were employed. The subject, standing, inhaled from a spirometer containing about 1 cc. of ethyl iodide in 300 liters of air. One hand was placed in water at 43° to 45°C.; and late in the experiment blood, equivalent to arterial blood in gas content (4) was obtained from a vein on the back of this hand. Respiration was recorded by a pointer, attached to the spirometer bell, writing on kymograph paper. Blood pressure and pulse rate were determined frequently. In the last experiments an electrocardiograph was used, recording from lead II. The subjects were instructed to tap with the foot whenever unusual sensations were noted, the time was then recorded on the record, and the signals interpreted after the experiment.

The subjects were healthy young men between the ages of 27 and 35. Three were doctors of medicine, one a physiologist. All were accustomed to the proceedings involved in this study. Subjects J. H. and H. H. had had syncopal attacks before, the others could not recall any.

After a brief period of adaptation, holding the hand in hot water caused no discomfort to the subjects. In all the entire skin became warm and moist. Some noted increased axillary perspiration. In one, who did not faint, there was generalized perspiration throughout the experiment.

RESULTS

Control experiments without faintness. In the experiments in which faintness did not occur no significant changes of blood pressure, pulse and respiration took place. Hence these records have not been given.

Experiments in which faintness occurred. Data collected in the six experiments are presented in the figures and described below.

I. Subjective sensations. Among the sensations of faintness experienced by the subjects were tinnitus, light headedness, vertigo, nausea, a sinking feeling in the abdomen similar to that produced by rapid descent in an elevator, and finally a feeling that near objects were becoming distant and indistinct. A feeling of apprehension often preceded and always accompanied more definite sensations. For convenience of expression these sensations will be referred to as faintness in the remainder of this paper.

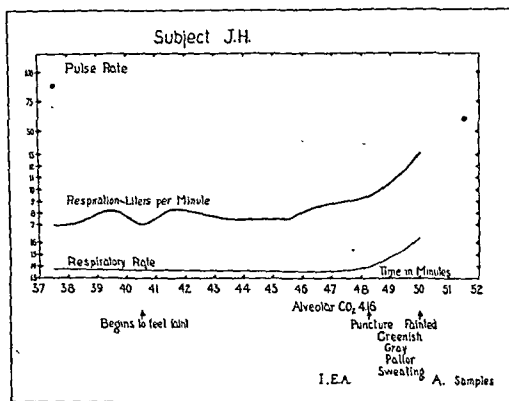
The arrows, placed on the figures under the supervision of the subjects, indicate gradual rather than sharp changes of sensation.

II. The circulation. The first estimations of cardiac output in experiments 3 and 4 were made after or during the preliminary rise of pulse rate but before the sensations of faintness, and changes in respiration and blood pressure had developed. In both cases the cardiac output was well within the normal range, much above the average normal basal level.

Unfortunately the data which could be secured during the actual period of faintness are insufficient to permit accurate calculation of cardiac output at that time, as recovery or collapse supervened too quickly to allow all requisite samples to be taken. Nevertheless, it

seems proper to assess the significance of such data as were obtained.

The samples of expired and of alveolar air secured during faintness in experiments 1, 3 and 4, together with knowledge of the concentration inhaled and of the respiration, permit the cardiac output



General explanation of figures. The results of pulse and blood pressure estimations have been placed at the time the determinations were begun. The scale for pulse and respiratory rates indicates the rate per minute; for volume of respiration liters per minute; for blood pressure the same scale is employed as for pulse rate, but it should be read as mm. of Hg. The samples of inspired, expired, alveolar, and rebreathed air, analyzed for ethyl iodide to estimate cardiac output, have been recorded under their initial letters at the time the sample was trapped. The content of alveolar samples represents the condition of alveolar air somewhat before trapping (3). The alveolar CO₂ has been recorded at the approximate time the percentage found was present.

FIG. 1. Experiment 1. At the end of the record the subject fell, was caught and carried to a nearby couch. He was unconscious for about a minute, recovered promptly, felt entirely well in about fifteen minutes.

to be calculated according to the method of Henderson and Haggard (5). The results are much above the average found in normal persons standing by Rosen and White (6), and are at or above the average calculated from our data in eighteen similar experiments (7). Also these results in experiments 2 and 3 are much above those calculated

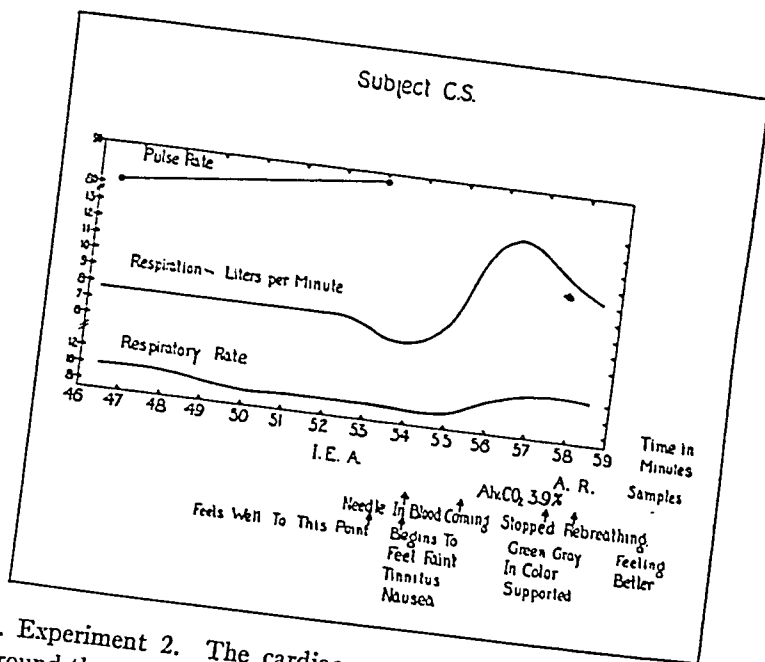


FIG. 2. Experiment 2. The cardiac output estimation was lost because of leakage around the mouthpiece at the height of symptoms.

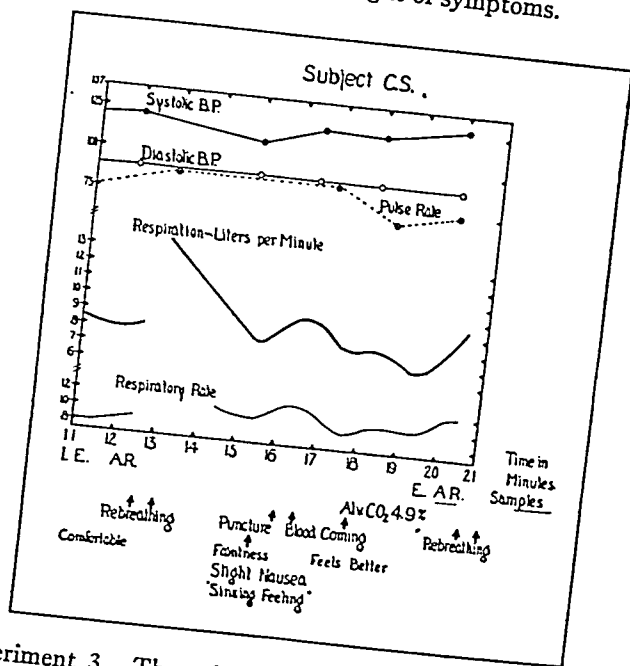


FIG. 3. Experiment 3. The subject, having become faint before, was apprehensive and excited. The pulse rose steadily from 66 to 88, the blood pressure changed from 118/80 to 120/90 during the 14 minutes preceding the period given in the figure. The first estimation of cardiac output indicated 6.9 liters per minute, the second 5.5 liters per minute. The unusually large increase in respiration following the first rebreathing was probably due to rapid accumulation of CO₂ from the high metabolism from excitement.

from experiments in which the same subjects stood without faintness (7). No similar control experiments on H. H. were performed. While we have little confidence in the figures for cardiac output thus obtained, it is obvious that the ethyl iodide in expired and in alveolar air during faintness bore the same relation to that inspired as occurs in persons

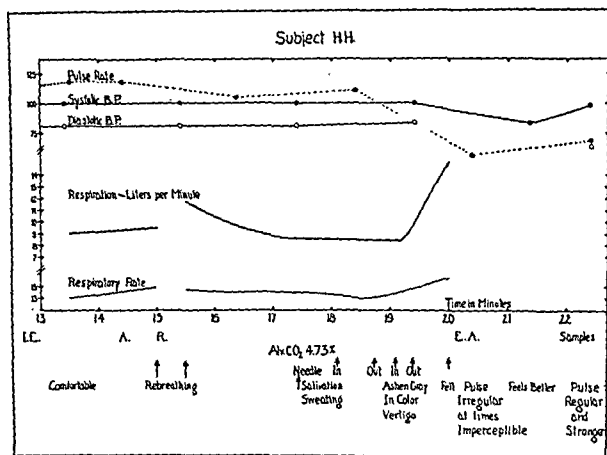


FIG. 4. Experiment 4. The pulse rose from 88 to 118, the blood pressure changed from 106/80 to 100/84 during the 16 minutes preceding the period given in the figure. The first cardiac output estimations indicated 5.1 liters per minute. The skin was punctured twice, no blood being obtained. At the end of the record the subject fell, was caught and laid flat on the couch. Some of the observers believed that he became unconscious, the subject thought not. He was fully conscious within a minute and continued to lie down for about 20 minutes.

with normal circulations. This suggests that the cardiac output during faintness was normal. But as the possibility of diminution of ethyl iodide in venous blood during faintness cannot be denied, we prefer to regard our evidence on the state of the cardiac output during these sensations as inconclusive.

Any speculation regarding the size of the cardiac output depends

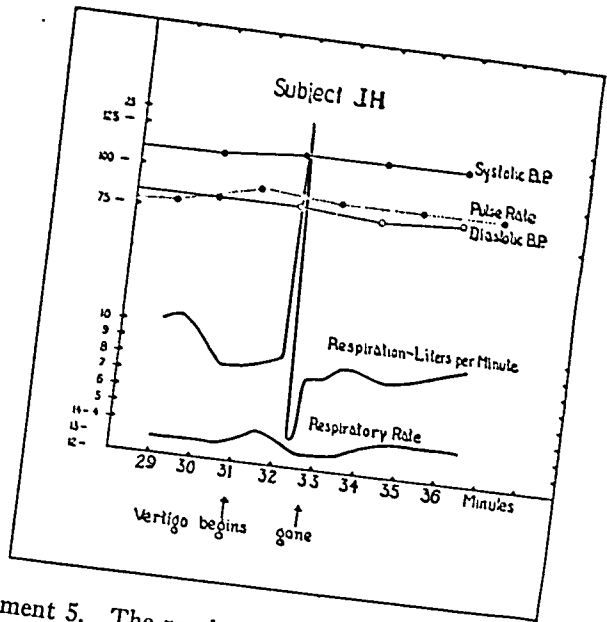


FIG. 5. Experiment 5. The respiratory change here recorded consisted of two very long deep sighing respirations, followed by several shallow breaths.

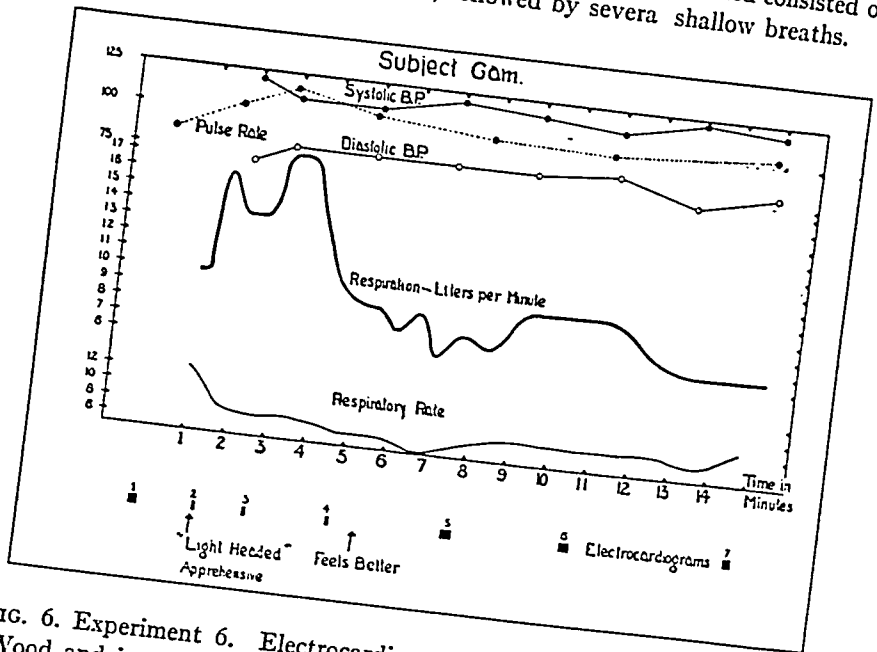


FIG. 6. Experiment 6. Electrocardiograms indicated, were taken by Dr. F. C. Wood and interpreted as follows: No. 1, normal, T wave upright 2.5 mm.; no. 2, T wave 1 mm.; no. 3, T wave less than 0.5 mm. isoelectric in one place; no. 4, T wave 0.5 mm.; no. 5, T wave 1 mm. at start rises to 2 mm. by end of record. No. 6 and no. 7, T wave 2 mm. No other changes except those of T wave.

The pulse rates recorded were obtained from the electrocardiograms.

on the assumption that the equilibrium of ethyl iodide between alveolar air and blood is not disturbed during faintness. In experiment 3 the agreement of the ethyl iodide content of "arterial" blood with that calculated from alveolar concentration and average normal distribution coefficient (6.1) demonstrates the existence of a normal equilibrium. The attempt to demonstrate it in experiment 2 failed because the fainting subject did not grasp the mouthpiece sufficiently to prevent leakage.

TABLE I
Data bearing on cardiac output before or during faintness

Experiment number	Ethyl iodide concentration				Respiration		Temperature correction	Cardiac output
	Inspired	Expired	Alveolar	Rebreathed	Last 5 minutes	Last minute		
	<i>mgm. per liter</i>	<i>mgm. per liter</i>	<i>mgm. per liter</i>	<i>mgm. per liter</i>	<i>liters per minute</i>	<i>liters per minute</i>		<i>liters per minute</i>
1 {a b	5.34	2.82	1.58 2.11		7.5	12.5	0.92	
2	5.77	2.50	1.84		7.7			
3 {a b	5.33	2.35 2.82	1.59 1.73	1.03 1.09	7.3 7.8	8.0	0.92	6.9
4 {a b	5.34	2.79 3.10	1.93 2.08	1.14	8.85	11.0	0.917	5.1

In calculating cardiac output the average normal distribution coefficient, 6.1 has been employed. The small correction for delay in taking the R sample (3) has been omitted as its value cannot be determined from the data.

Therefore we have obtained no evidence of a diminished cardiac output before or early in the process of fainting. Later when pulse rate and blood pressure had fallen markedly, a diminished cardiac output seems highly probable. We have obtained no evidence on this point.

In the five experiments in which enough counts were made, the pulse rate always increased before or early in the sensations of faintness, while the control experiments showed no similar changes. Later in the experiments the pulse rate always fell after recovery (experiments 3, 5, and 6) or after syncope (experiments 1 and 4). In ex-

periment 4 the rate during syncope was one-half that recorded before it and the pulse was irregular and at times almost imperceptible. In experiment 1 the pulse during syncope was slow but regular and strong.

The changes of blood pressure noted before syncope were so small that we are not confident of their significance. After syncope a profound fall in both systolic and diastolic pressure occurred. In experiment 4 the latter fell so far that the observer, eager to get another systolic reading, did not wait for the cuff to deflate far enough to determine the diastolic level. Recovery was accompanied by a return towards normal.

The marked cadaveric pallor, observed in experiments 1, 2, and 4 at the height of the symptoms, should be attributed to peripheral vaso-constriction. It appeared late in the process, immediately before collapse. In other experiments the symptoms were milder and pallor did not appear.

Blueness of the lips was noted just before collapse in experiment I. In other experiments the greenish color was doubtless due to the combination of cyanosis and pallor.

In experiments 2 and 3 samples of blood, obtained from a vein on the back of the hand in hot water during the sensations of faintness, were the color of arterial blood. In experiment 3 this blood contained 18.5 volumes per cent of oxygen, and was 90.4 per cent saturated. The analyses were performed in duplicate in the apparatus of Van Slyke and Neill (8). No blood was secured from the other subjects.¹

III. Changes of respiration. A great increase in minute volume during faintness was characteristic of all our observations. In three experiments the increase started coincidentally with the beginning of the sensations, while in two the sensations preceded the increase. In Experiments 2 and 3, minute volume is recorded as diminishing before the sensations passed away, although in experiment 2 this may

¹ With the assistance of Dr. Francis C. Wood frequent electrocardiograms were made in two cases. In one subject (experiment 6) the T wave, 2 mm. high before faintness, diminished during the sensations till it was less than 0.5 mm. high and returned to its previous level as recovery occurred. However, later in the same experiment a similar diminution of the T wave took place without any faintness or other change in our records to account for it. Therefore these observations throw no light on the physiology of fainting.

be an artifact because the subject was so weak that his cheeks ballooned on each expiration and leakage of air around the mouthpiece took place.

When syncope occurred and the subject released the mouthpiece the respiratory record ceased. The violent movements of the chest, visible during the hyperpnea, were not seen after the subject was horizontal.

The rate usually changed in the same direction as the minute volume but the change often began later. In experiment 5 the rate fell as minute volume increased, but this continued only for a very brief period. In no experiment did the increase in rate account for the increased minute volume, which was primarily caused by the great increase in depth. During syncope no accurate counts were made, but the rate appeared to become slower.

As the alveolar air was collected by the repeated addition of small amounts to a large sampling tube, the gases found by analysis represented the content of the alveoli about two and a half minutes before the collection was completed. Therefore, if the respiration was increasing, the percentage of CO_2 in the alveolar air when the sampling tube was closed must have been less than that found by analysis of the sample. The alveolar CO_2 at syncope must have been lower than the figures here reported.

It is obvious that the alveolar CO_2 found was abnormally low in all cases in which syncope occurred. Identical experiments without faintness showed alveolar CO_2 contents over 1 per cent higher for C. S. and H. H., from 0.1 to 0.7 per cent higher for J. H. It is interesting that C. S., so faint that he had to be supported, recovered during a rebreathing period of 1 minute, 25 seconds duration, though he continued to stand upright.

While hyperventilation with blowing off of CO_2 will cause low blood pressure in animals (9, 10), increasing hyperventilation of the grade recorded in our subjects is not sufficient to cause much change of blood pressure, or to produce symptoms in normal subjects (11). On the other hand subjects who complain of "light headedness" when breathing deeply are occasionally encountered (12). Therefore the lowering of blood CO_2 , though not the cause of syncope, may well be a factor in the production of the symptoms.

DISCUSSION

Relation of venipuncture to the production of symptoms. Only in the case of C. S. (experiments 2 and 3) did the puncture of the vein precipitate the symptoms. In experiment 1 the symptoms preceded the puncture by eight minutes and the subject was so faint that he felt no pain and hardly realized the puncture was being performed. In experiment 4 the symptoms began about one-half minute before puncture, during preparations to perform it. In the other experiments the faintness appeared and passed off so long before the puncture that the latter is not shown in the figures. Obviously the symptoms were not caused by a reflex from the discomfort of venipuncture in most of our experiments, although in two, such an explanation is possible.

Picture obtained by combining our results. The initial physiological changes of our experiments were so similar that they suggest a uniform response to similar pathological conditions. The differences of response found later may be attributed to differences in intensity of this condition, as in some cases it soon terminated in recovery, in others it progressed to collapse. Therefore it seems proper to combine our observations and so obtain a description of the process of fainting more complete than in the best single experiment (experiment 4). This description is as follows.

After a period of several minutes in which the pulse rate slowly rises the sensations of faintness appear, soon followed by a steadily increasing volume of respiration with increased respiratory rate. Changes of blood pressure are small and the cardiac output is not reduced at this stage. Recovery may follow or increasing severity of sensations, hyperpnea, greenish pallor, sweating, and perhaps cyanosis appear, soon followed by loss of consciousness and collapse. Decreased respiration, and a profound fall in blood pressure and pulse rate are now observed.

Similarity of our observations to effects of cerebral anemia. It has long been known that faintness and unconsciousness follow surgical ligation of carotids in unanesthetized patients, that these symptoms may be produced by compression of the carotid arteries (13), and that their relief promptly follows lowering of the head. It has been universally agreed that faintness and syncope are due to cerebral anemia.

It is proper to examine our data and ask whether the physiological changes found are those known to follow cerebral anemia in animal experiments. In figure 7 are shown the changes following cerebral anemia produced by elevation of the head or constriction of the carotids in anesthetized cats prepared by Dr. Carl F. Schmidt. The hyperventilation followed by apnea, the initial slight changes of blood pressure without change in pulse rate, later followed by great slowing of the heart and a fall in blood pressure, are all consistent with the observations on our subjects. Other investigators (14) have reported similar results and have also shown that the cardiac slowing disappears after section of the vagi (15). The similarity of these responses following cerebral anemia to the changes we observed during faintness is obvious. Therefore we agree that cerebral anemia is the probable cause of faintness and syncope. However, it should be pointed out that the results pictured in figure 7 are by no means always obtained and in many experiments cerebral anemia causes a rise of blood pressure followed by cardiac slowing with fall of blood pressure in animal experiments (16).

Possible causes of cerebral anemia in our experiments and in other reported cases. Concerning the cause of the cerebral anemia there is difference of opinion and without doubt difference of physiological mechanism is to be expected in different cases.

In our experiments cardiac slowing followed both an increase of pulse rate and the onset of sensations of faintness. Therefore reflex vagus stimulation, demonstrated to be the cause of syncope in Laslett's case (2) and suggested by Cotton and Lewis (1) as the explanation of fainting attacks in soldiers with irritable hearts, was not the primary cause of symptoms in our subjects.

We have obtained no evidence of diminished cardiac output or fall of blood pressure before the development of sensations. The conception of Field and Bock (17) and Turner (18, 19) that faintness on prolonged standing is due to cerebral anemia secondary to decreased cardiac output from diminished venous return does not apply to all our subjects. But undoubtedly a low cardiac output must be considered a predisposing cause of cerebral anemia as it must greatly increase the difficulty of maintaining an adequate circulation to the brain. This should be considered a factor in subject J. H., whose

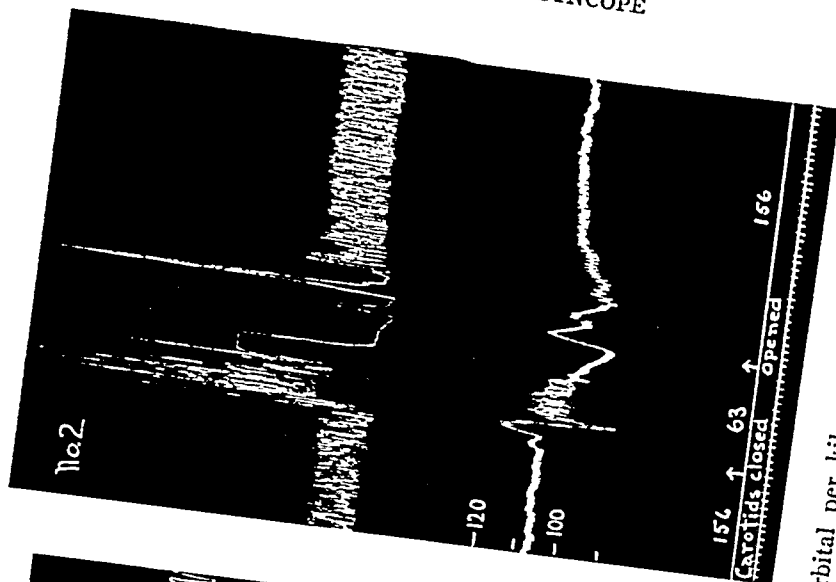
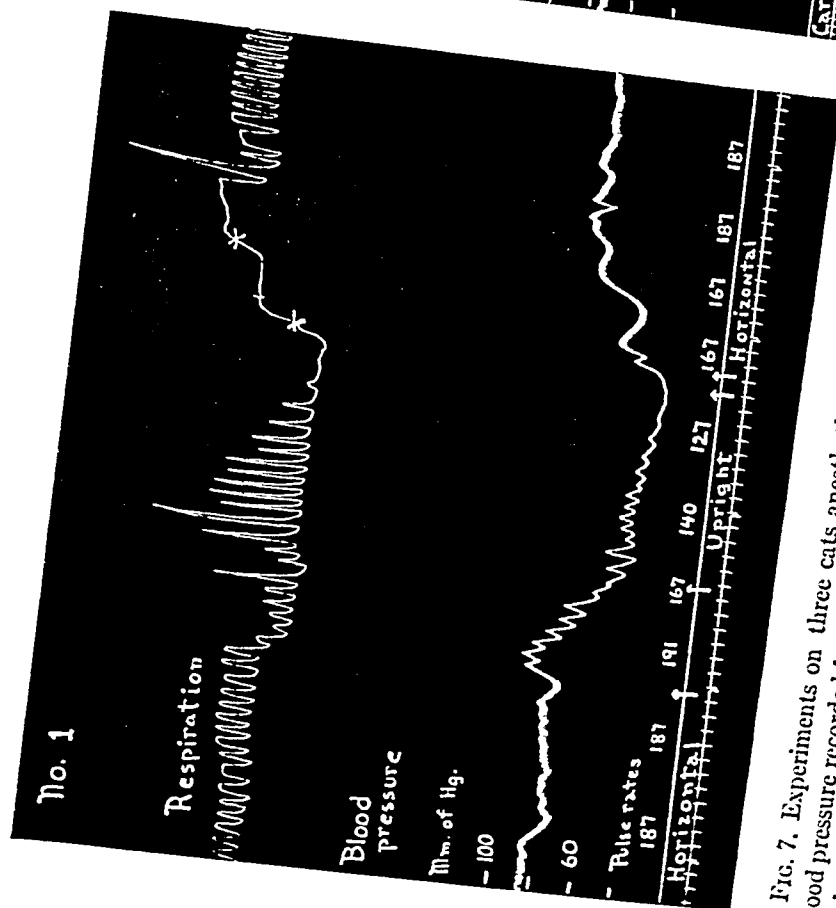


FIG. 7. Experiments on three cats anesthetized with 0.4 gram of sodium barbital per kilogram. Tracheal cannulae. Blood pressure recorded from carotid cannula in nos. 2 and 4, femoral cannula in nos. 1 and 3. Respiration recorded from an inflated bag around chest and tambour in nos. 1, 3, and 4; from Cushny plethysmograph in no. 2. The time records show five second intervals.

No. 1. Right vagus and both cervical sympathetics cut in a previous experiment. Vertebrae open. Cerebral anemia caused by raising animal's head to upright position. The change of level in the respiratory tracing is an artefact.

No. 2. Vertebral arteries tied previously. Cerebral anemia from simultaneous closure of carotids.

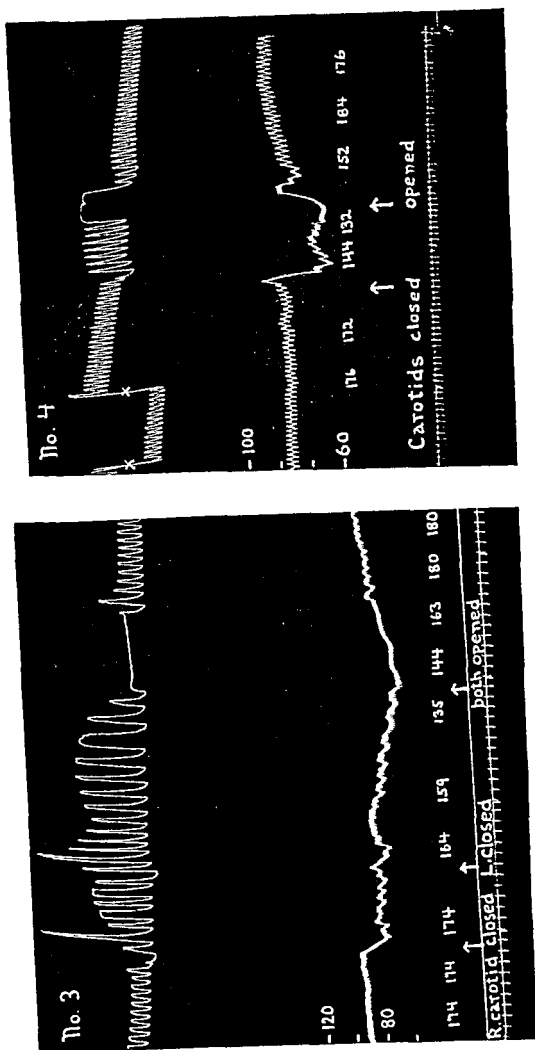


FIG. 7. See page 572 for explanation.

No. 3. Same animal as no. 1. Carotids closed separately, released together.

No. 4. Vertebrals open. Nerves intact. Cerebral anemia from closure of both carotids. After experiments illustrated in 1 and 3 the remaining vagus was cut. After this cerebral anemia caused no slowing of pulse and usually a rise of blood pressure. Stimulation of central end of sciatic still caused a good rise of blood pressure.

cardiac output standing is constantly lower than when lying (7). This subject gave a history of occasional fainting after trivial causes. Several other subjects whose cardiac output standing resembled that lying (7) had no such history.

The three observations that faintness occurred when the blood pressure was normal deserve emphasis. This indicates the presence of sufficient energy to raise the blood to the brain if the cerebral vessels had been normal. Therefore, if the conception that cerebral anemia caused the symptoms is correct, this anemia cannot be wholly attributed to changes in the circulation outside of the vessels supplying the brain, and cerebral vasoconstriction is suggested. The old explanation (20) that cerebral vessels are largely passive and that cerebral anemia is precipitated by failure of vasomotor adjustment in the rest of the circulation cannot be applied to our experiments. Active contraction of cerebral vessels has been demonstrated (21) but knowledge of this subject has not advanced far enough to warrant discussion of cerebral vasoconstriction as a cause of syncope.

SUMMARY

Physiological changes preceding and coincident with sensations of faintness have been studied in six experiments in four healthy male subjects. Observations made included the subject's appearance, his cardiac output, pulse, blood pressure, respiration (graphic record), alveolar CO_2 and blood oxygen content. In one experiment an electrocardiograph was employed.

The records obtained are sufficiently detailed to demonstrate the order in which the symptoms developed and to permit correlation of physiological changes with sensations.

Before the sensations of faintness the pulse rate rose, but the respiration, blood pressure and cardiac output remained normal. At or soon after the beginning of faintness the volume of respiration began to increase and the alveolar carbon dioxide tension diminished, but at this stage normal blood pressure was found. Later, during syncope, a profound fall of pulse rate and blood pressure occurred.

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THE EXCRETION OF INTRAVENOUSLY INJECTED BILIRUBIN AS A TEST OF LIVER FUNCTION

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The liver plays such an important rôle in the animal body that the study of methods for testing its functional ability has attracted and still attracts considerable interest. As this gland is believed to be the site of internal as well as of external secretions, it is probable that dissociated functional disturbances may arise when it is acted upon by an injurious agent. At least theoretically, therefore, an adequate test for liver function ought to include the study of all of its multiple activities. It is likely, however, that some of these functions are more susceptible to the action of noxious agents and are readily damaged when other functions are still unimpaired. Such appears to be true as regards the ability of the liver to excrete bilirubin. The important place which the Van den Bergh reaction and the determination of bilirubinemia have taken in the study of liver disease indicates the trend of current opinion.

It is, however, well recognized that there are conditions of mild liver injury where the bilirubinemia is not increased, either because the liver cells are still able to excrete the normally circulating bilirubin or because there is a diminished production of the pigment. In either case the insufficiency of the liver might be demonstrated by injecting an additional amount of the pigment and studying the rate of its excretion. Working on this theory, Barron and Rich (1) have recently been engaged in the study of bilirubin excretion in dogs with the intention of applying the method later to clinical use. Meantime reports have been published in Germany by Von Bergmann (2) and his associate, Eilbott (3) who have applied the bilirubin excretory power of the liver to the study of a variety of hepatic disorders, particularly cirrhosis, chronic passive congestion of the liver, cholecysti-

tis and miscellaneous cases of jaundice. These authors injected 70 mgm. of bilirubin dissolved in 10 cc. of 5 per cent NaOH estimating the blood concentration at measured time intervals thereafter.

THEORETICAL BASIS FOR THE BILIRUBIN EXCRETION TEST

From the considerable literature devoted to the problem of the site of bilirubin formation it can be concluded that there is no evidence that the polygonal liver cells are concerned with the formation of bilirubin. In all probability the cells of the reticulo-endothelial system are actively concerned in the formation of the pigment through hydrolysis of the blood hemoglobin and possibly of related pigments.

The site of bilirubin excretion has been less subject to discussion. As early as 1874, Tarchanoff (4) studied the excretion of intravenously injected bilirubin in dogs, determining the pigment in the bile and urine by means of fistulas of the common bile duct and ureters. He reported that all of the injected bilirubin was found in the bile and none in the urine. These observations were confirmed by Vossius (5) in the same animal and by Beckmann (6) in rabbits. They used the Gmelin test for detecting bilirubin in the urine. These observations are correct in their essential conclusions. The liver of the dog possesses such an enormous ability to excrete bilirubin that even though there is no renal threshold for bilirubin, the amount secreted through the kidneys is at its maximum only one per cent of the injected bilirubin. In man the kidney does not take any part in the excretion of the pigment, under the conditions of our experiments, since we have studied only individuals with blood serum showing an indirect Van den Bergh reaction. Although the bilirubin solution, which is injected intravenously, gives a direct Van den Bergh reaction, as soon as it enters the blood stream it is adsorbed by the proteins of the blood serum and the Van den Bergh reaction becomes indirect, exactly in the same manner as occurs when a solution of bilirubin is added *in vitro* to serum (Harrop and Barron (7)). It is well known that bilirubin giving the indirect Van den Bergh reaction is not excreted through the kidneys. It can be argued that the injected bilirubin may be phagocyted by the cells of the reticulo-endothelial system in the same manner as occurs when foreign pigments are injected into the blood stream. The ob-

servations of Kanner (8) are against such an assumption. Studying systematically the reticulo-endothelial system in different types of jaundice, Kanner (8) found that only in cases of complete obstructive jaundice was there any storage of bilirubin within the Kupfer cells; in conditions of partial obstructive jaundice and non-obstructive jaundice he never found evidence of bilirubin storage whether in the Kupfer cells or at any other place in the reticulo-endothelial system.

The studies which have been briefly summarized above indicate that injected bilirubin is not excreted through the kidneys, that it is not retained by the cells of the reticulo-endothelial system; and that it is totally excreted by the liver. The rate of excretion of bilirubin injected intravenously may therefore be employed as a rational test for liver function.

SOLUBILITY OF BILIRUBIN

It is well known that bilirubin is soluble in aqueous solvents, only when they are highly alkaline. Kerppola and Leikola (9) have studied the solubility of bilirubin at different hydrion concentrations, mixing solutions of bilirubin in chloroform with various buffers. They found that the pigment remained in the chloroform solvent from pH 0.0 to pH 6.5; that it is entirely dissolved in the buffer at pH 12 to pH 14 and that from pH 7 to pH 11.5 some of the pigment remains dissolved in chloroform while another portion goes into the buffer. We have studied the solubility of bilirubin in aqueous solvents at different hydrion concentrations, using in all the experiments the same bilirubin concentration, namely, 0.4 per cent. Bilirubin at such concentration can be kept entirely in solution when using solvents having a pH of about 11.0 provided the temperature of the solvent has been previously raised to 80°C. Once the bilirubin is in solution it can be kept dissolved until the pH is lowered to 9.4 by addition of appropriate buffers.

TECHNIQUE OF THE BILIRUBIN EXCRETION TEST

The technique which we have employed is as follows: The bilirubin used was obtained from the Eastman Kodak Company and was used without previous sterilization at a concentration of 1 mgm. per

kilogram of body weight.¹ It was dissolved in 15 cc. of 0.5 M Na_2CO_3 previously boiled, after the solution had cooled to about 80°C. The final pH of the solution at 20°C. was 11.08 (as determined by the hydrogen electrode). A control sample of blood was taken before the injection. After the injection, blood was taken at the following intervals: 5 minutes, 30 minutes, 2 hours and 4 hours. The blood was received in test tubes containing 3 drops of 10 per cent of potassium oxalate which was previously dried. It was then kept in the dark in the ice box until the final collection was completed.

Determination of bilirubin. In a previous review of the different methods used for the estimation of blood bilirubin (Harrop and Barron (7)) it has been stated that the Van den Bergh method is the most reliable and accurate of the clinical methods. When determining small amounts of the pigment however the colorimetric readings are extremely uncertain and unreliable. We have therefore used Ernst and Förster's method (10) which is performed essentially as follows: The plasma is precipitated by acetone, which is used at different concentrations according to the amount of bilirubin present in the plasma. After shaking it is centrifuged and filtered, and the filtrate read at once in a colorimeter against a standard composed of potassium dichromate, 1 to 6000, which had previously been standardized against a solution of bilirubin. By this method one determines both the bilirubin and the unknown amount of lipochromes present in the blood plasma. It seems safe to assume however that there can be no change in the lipochrome concentration during the performance of the test. The error therefore remains constant and is removed from consideration by subtracting the figure found as the bilirubin value in the control sample from the figures obtained from the other samples. Van den Bergh has shown (11) that bilirubin giving an indirect reaction is not adsorbed by the alcoholic precipitate of the serum proteins; the same thing applies to the acetone precipitate. As the Van den Bergh reaction in all the samples is indirect (provided the original

¹ We have recently had difficulty in obtaining bilirubin for injection from the Eastman Kodak Co. owing to temporary difficulties in their process of purification which they state will shortly be overcome. Meantime, Dr. L. J. Soffer has employed Bilirubin "Homburg," obtainable from Chemisch-Pharmazeut A. G., Bad Homburg, Germany, with equally satisfactory results, in this clinic.

control sample is indirect) there is no loss of bilirubin due to the precipitation of proteins.

The rate of bilirubin excretion was plotted according to Von Bergmann and Eilbott's method, i.e., the bilirubin content of the sample taken five minutes after the injection minus that of the control sample, was considered as containing 100 per cent of the injected pigment. The percentage retention of bilirubin in the other blood samples was then calculated after previous subtraction of the bilirubin contained in the control.

THE EXCRETION OF BILIRUBIN BY THE LIVER IN NORMAL INDIVIDUALS

When bilirubin is injected intravenously into normal individuals at the concentration given above, i.e., 1 mgm. per kilogram of body weight, it is totally excreted in from 2 to 4 hours. Table 1 and figure 1 (dotted lines) show the rate of excretion of the pigment in normal persons. As is there shown, in the majority of cases the bilirubin was totally excreted within 2 to 3 hours after the injection. In some cases at the end of 3 hours, a slight retention still existed, but in these cases all the pigment had been excreted at the end of 4 hours. We may therefore conclude that 4 hours is the maximum limit for the total excretion of bilirubin in normal individuals. Whenever bilirubin retention is still present at the end of 4 hours we may consider that the power of the liver to excrete the pigment has been impaired.

THE APPLICATION OF THE BILIRUBIN EXCRETION TEST TO PATHOLOGICAL CONDITIONS

Our interest in the clinical application of this liver function test has been confined to cases of slight liver insufficiency which could not be detected by either the Van den Bergh test or by the use of the levulose or bromsulphalein tests. The introduction of an additional test for hepatic function is scarcely justified unless it yields information to the clinician which is not otherwise obtainable.

An interesting condition in which liver damage may be possible but has never as yet been clearly demonstrated is severe anemia. Rich and Resnik (12) observed that in pernicious anemia, as well as in experimentally produced anemias, the cells about the efferent veins of each liver lobule may be damaged in a manner often indistinguishable

from that accompanying chronic passive congestion. They advanced the suggestion that the damage to the liver cells may be due to poor oxygenation. It was of interest to study the bilirubin excretory power of the liver in such cases. There is in pernicious anemia a hyperbilirubinemia which is considered by many authors as of hemolytic nature and due solely to the increased red cell destruction. Secondary anemias usually show a hypobilirubinemia. The present test has been

TABLE 1
The serum bilirubin retention in individuals without demonstrable liver injury

Case number	* Per cent of bilirubin retention in blood serum				Diagnosis and remarks
	30 minutes	2 hours	3 hours	4 hours	
1	35.0	0.0			Convalescent from rheumatic fever normal temperature 18 days
2	32.8	0.0			Gastritis
3	16.5	6.4	0.0		Recovered from lobar pneumonia normal temperature for 3 weeks
4	38.7	9.0	0.0		Neurasthenia
5	36.5	9.6	0.0		Sulphemoglobinemia
6	28.2	8.6	0.0		Sulphemoglobinemia
7	41.0	6.0	0.0		Chronic arthritis
8	36.0	4.0	0.0		Normal
9	45.5	26.8	0.0		Polycythemia vera
10	31.7	19.0	2.7	0.0	Polycythemia vera
11	53.0	9.0	4.0	0.0	Hernia
12	60.0	11.2	5.0	0.0	Gastric ulcer

Bromsulphalein and Levulose Tolerance Tests showed normal function in all of these cases.

* Percentage calculated on basis of amount present in blood 4 minutes after injection considered as 100 per cent.

used clinically on five patients with primary anemia and two patients with secondary anemia. In all of these cases other tests for hepatic function were also performed: (a) The Van den Bergh reaction, which was always indirect; (b) the quantitative estimation of bilirubinemia which never was beyond 1 mgm. per cent; (c) the levulose tolerance and bromsulphalein tests which in all of these cases yielded no evidence of liver damage. Table 2 and figure 1 show that in all, except

one case of pernicious anemia, there was a definite retention of bilirubin at the end of four hours, indicating the presence of a functional disturbance of the liver.



FIG. 1

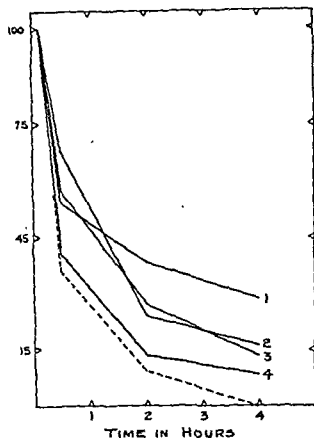


FIG. 2

FIG. 1. BILIRUBIN EXCRETION CURVES IN CASES OF CHRONIC ANEMIA

Ordinates, per cent of bilirubin excreted. Abscissa, time in hours. Dotted line—normal curve. Full lines—cases of anemia.

FIG. 2. BILIRUBIN EXCRETION CURVES IN MISCELLANEOUS CASES OF LIVER DAMAGE

Ordinates, per cent of bilirubin excreted. Abscissa, time in hours. 1, typhoid fever, presumably slight infectious hepatitis; 2, cirrhosis of the liver; 3, post-arsphenamine jaundice (Clinically recovered); 4, carcinoma of the liver. Dotted line—normal curve.

In table 3 are given the results obtained in a number of pathological conditions, including cirrhosis of the liver, acute infectious disease in which liver damage may be suspected, and post-arsphenamine jaundice some months after clinical recovery. The demonstrations of definite liver damage in typhoid and other fevers, without clinical

TABLE 2
The bilirubin excreting power of the liver in chronic anemias of various types

Case number	Per cent* of bilirubin retention in blood serum			Van den Bergh†	Bromsulphalein test‡		Levulose test	Diagnosis and remarks
	30 minutes	2 hours	4 hours		per cent	interpretation		
13	31.7	20.7	13.4	Indirect	30-5	Normal	Negative	Pernicious anemia, duration about 1 year; R.B.C. 1.89 million; hemoglobin 55 per cent
14	47.3	20.0	12.3	Indirect	30-0	Normal	Negative	Pernicious anemia, duration about 2½ years; R.B.C. 2.2 million, previously 0.8 and 1.0; hemoglobin 46 per cent
15	42.3	17.0	10.4	Indirect	20-0	Normal	Negative	Pernicious anemia, duration 4½ years; R.B.C. 1.3 million; hemoglobin 36 per cent
16	49.5	25.8	8.9	Indirect	35-5	Normal	Negative	Pernicious anemia, duration 15 months; R.B.C. 1.2 million; hemoglobin 29 per cent
17	50.6	21.6	0.0	Indirect	30-0	Normal	Negative	Pernicious anemia, duration 3 years; R.B.C. 3.5 million; hemoglobin 86 per cent
18	42.6	18.8	15.0	Indirect	20-0	Normal	Negative	Secondary anemia, epistaxis; R.B.C., November 1, 1.48 million; hemoglobin 31 per cent; November 20, 2.23 million; hemoglobin 45 per cent
19	55.0	25.2	9.4	Indirect				Secondary anemia; R.B.C. 2.0 million for 1 month previous to test

* See note in previous table.

† Van den Bergh reaction in all cases less than 1 mgm. per cent bilirubin retention.

‡ Numerals indicate percentages of dye present in blood serum at end of 5 and 30 minutes after injection.

TABLE 3
Various types of liver injury—bilirubin excretory power of the liver

Case number	Per cent* of bilirubin retention in serum			Van den Bergh	Plasma bilirubin	Bromsulphalein test†		Levulose test	Diagnosis and remarks
	30 min-utes	hours				per cent	interpretation		
		2	4						
20	67.0	24.1	16.3	Indirect	0.48	25.0	Normal	Slight increase	Cirrhosis of the liver (syphilis)
21	63.0	33.6	10.4			50-20	Slight increase	Negative	Laenec cirrhosis
22	39.0	22.3	12.8	Indirect	0.60	60-40	Marked retention	Negative	Laenec cirrhosis
23	40.3	13.8	7.9	Indirect	0.35	30-0	Normal	Negative	Large liver, carcinoma metastases confirmed only at operation. Previously thought to have no liver disease
24	57.4	27.2	14.0	Indirect	0.51	35-0	Normal	Negative	Post-arsphenamine jaundice, 1 year previous. Clinically recovered completely
25	44.2	28.8	8.7	Indirect	0.59	25-5	Normal	Negative	Infectious hepatitis? Lobar pneumonia. Eighth day of disease. 103.5 temperature
26	53.2	38.4	27.9	Indirect	0.55	30-5	Normal	Negative	Typhoid fever. Temperature 104.0. Third week
27	42.7	36.7	5.5	Indirect	0.60	30-0	Normal	Negative	Malaria inoculata after 8 paroxysms of chills and fever

* See note in table 1.

† Numerals indicate percentages of dye present in blood serum at end of 5 and 30 minutes after injection.

EXCRETION OF BILIRUBIN

evidences of jaundice or of any other functional disturbance is of considerable interest, as is that of continued liver disturbance in post-arsphenamine jaundice long after clinical recovery has apparently taken place. It seems indeed possible that such damage may be permanent after infections or intoxications in which at the time, or subsequently, it is entirely unsuspected, and that a systematic study of such mild degrees of liver damage may explain many liver disorders in later life, the etiology of which is quite obscure at present.

We may call attention to the fact that the bilirubin excretion was normal in the two cases of polycythemia vera studied (cases 9 and 10). This has been a matter of surprise to us and we are not prepared to state, as a general rule, that no retention occurs in this disease without further study. In both of these cases, which we have followed for several years, due to phenyl hydrazine and x-ray therapy, the blood counts were not elevated, and at the same time of these bilirubin studies the liver in neither individual was enlarged on physical examination. Splenomegaly was present in both patients.

SUMMARY AND CONCLUSIONS

The introduction by Van den Bergh of a method of estimating blood bilirubin and his discovery of the difference in behavior of serum bilirubin towards the Ehrlich diazo-reagent contributed greatly to our knowledge of liver pathology. Cases of liver damage could easily be followed in their evolution by the use of these simple methods. But there still remain cases of liver insufficiency where there is no retention of the pigment because the liver, although damaged, can still excrete normally the circulating bilirubin. It is logical to assume that these slight liver insufficiencies may be recognized by increasing the blood bilirubin concentration and testing the power of the liver to excrete this artificially produced hyperbilirubinemia. The experiments above reported prove that such is the case. We have chosen for our studies selected cases of slight liver injury where all the commonly used methods failed to give evidence of damage of the liver and in all these cases we have found that it was possible to recognize an insufficiency of the liver as shown by delayed bilirubin excretion. It seems justified to conclude that study of the bilirubin excretory power of the liver is the most delicate method so far proposed for testing the functional capacity of this organ.

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CHANGES IN BLOOD VOLUME IN PATIENTS WITH EDEMA OF RENAL ORIGIN

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The purpose of the present study was to apply the carbon monoxide method to the investigation of the circulating blood volume in cases of renal edema, and to follow by this means the changes in the blood volume of patients during the disappearance or reappearance of their edema. The problem appeared to us one of particular interest in view of the fact that the investigations that have been published so far have, with few exceptions, been carried out by means of the dye method; moreover the results that have been obtained by that method have shown remarkable differences in the hands of different observers, two groups of workers getting with it results not only opposed, but diametrically opposed, to one another.

In looking through the literature it appears that three cases reported by Plesch are the only ones in which the blood volume determinations were made by methods other than the dye method. In 1909 (2) with his infusion method he found in a case of renal edema of the most extreme degree a blood volume that was only half the normal. In 1922 (1) with the carbon monoxide method the same author found in two cases of renal edema a small volume which increased as the edema became less.

In 1924 (3) Linder and Lundsgaard found in cases of renal edema normal plasma volume using the dye method.

In 1926 (4) Darrow reported four cases of nephritic edema—three children and one adult in which the blood volume had been determined by the dye method. In all four cases the blood volume was diminished, whether calculated as volumes per unit of actual weight or as volumes per unit of edema-free weight. In two of the cases—

both children, diagnosed nephrosis with no elevation of nonprotein nitrogen or blood pressure—subsequent determination during the disappearance of the edema showed a great increase in the blood volume which was mainly in the plasma fraction.

In 1927 (5) a number of blood volume determinations by the dye method in acute and subacute glomerular nephritis were reported by Rusznyák who found the blood volume slightly diminished.

In 1925 (6) Brown and Rowntree, using their dye method, had already investigated four cases of nephrosis and four cases of subacute glomerular nephritis; and in 1928 (7) the same authors published an additional series of nine cases of nephrosis and twelve cases of glomerular nephritis. They concluded that whereas the cases of glomerular nephritis gave in general low or low-normal volumes with normal plasma volumes but decreased cell volumes, the cases of nephrosis gave, when uncomplicated by anemia, normal volumes and, when anemia was present, volumes that were somewhat increased both in respect of the whole blood and of the plasma. They differentiated also between these two groups in regard to the mechanism of diuresis: in glomerular nephritis disappearance of the edema was generally accompanied by a further reduction of the blood volume due mainly to a fall in cell volume in consequence of the progressive anemia; while in nephrosis no constant change was observed during diuresis either in cell or plasma volumes.

The point of greatest interest in the foregoing results is the remarkable divergence among them: on the one hand Brown and Rowntree find in nephrosis normal or increased plasma volumes with little or no change during diuresis; on the other hand Darrow obtains volumes markedly decreased in respect of both whole blood and plasma, with great increase in both during diuresis. The two cases of Plesch give low volumes with increasing volume during diuresis. One cannot be sure from his data whether they were cases of nephrosis or glomerular nephritis, but in either case they behave during diuresis in the opposite way to the cases of Brown and Rowntree. Yet it must be realized that there are two facts which may possibly account for the discrepancies: first, that Darrow worked with children and Brown and Rowntree with adults, and secondly that Brown and Rowntree used the dye method while Plesch used the carbon monoxide method.

METHOD

In our own studies on the blood volume in cases of renal edema we have used throughout the modification of Haldane's carbon monoxide method described by Chang and Harrop (8); in fact we have used the actual apparatus which they constructed. We have, however, introduced the precaution of standardizing the posture of the patient (in view of the work of Thompson, Thompson and Daily (9)), keeping them lying in bed for at least twelve hours prior to each determination.

In all cases the carbon monoxide content of the blood was determined in a sample drawn before rebreathing of the gas was started, and this figure was subtracted from the carbon monoxide content after rebreathing, in order to obtain the percentage of carbon monoxide that was present as a result of the inhalation alone.

TABLE 1
Blood volumes of eight normal adults in the recumbent posture

	Whole blood, cc. per kgm. ideal weight	Plasma, cc. per kgm. ideal weight	Cells, cc. per kgm. ideal weight
Average.....	71.4	40.5	30.9
Range.....	61.8-82.3	35.6-47.2	26.3-36.4

In eight normal adult males the blood in cc. per kgm. ideal weight was found by this method and with the same apparatus and technique to have the average figures and range of variation in the recumbent posture as shown in table 1.

CASES AND RESULTS

Five cases were studied. On all but one of them several blood volume determinations were made. In every case the actual blood volume was calculated, and also the blood volume in cubic centimeters per kilogram of ideal weight. The ideal weight was employed because of the large amount of edema present.

Case 1. M. L., number 17,816. Age 42 years, Italian housewife. Diagnosis: anasarca; transient albuminuria; toxemia of pregnancy; nephrosis.

She was admitted April 10, 1928, one month after her seventh normal delivery, with general anasarca which had started to appear during the last few weeks of

pregnancy. On admission there was considerable edema of the legs with ascites. There was no anemia. The urine showed a trace of albumin, no casts and a very occasional red cell; the specific gravity was 1020 and the reaction acid. The blood pressure was 160/100 mm. Hg. The nonprotein nitrogen had the normal figure of 33 mgm. per 100 cc. As shown in table 2 the cholesterol content of the plasma was raised while the serum protein had a remarkably low value. There was an inverted albumin-globulin ratio of 0.47 to 0.53.

On a high protein diet the serum protein rose rapidly and at the same time the albumin in the urine, which had previously been only a trace, increased to a large daily output.

Finally the serum protein reached a normal figure of between 6 and 7 grams per 100 cc., the albuminuria ceased and the edema disappeared. The blood pressure also fell, and was 118/63 mm. Hg on her discharge six weeks after admission.

TABLE 2
Variations in blood volume with disappearance of edema. Case 1

Date	Weight	Hemo- globin	Total protein	Choles- terol	Hema- tocrit	Volume			Volume per kgm. ideal weight		
						Whole blood	Plasma	Cells	Whole blood	Plasma	Cells
1928	kgm.	per cent	grams per 100 cc.	mgm. per 100 cc.	percent- age of cell volume	liters	liters	liters	cc.	cc.	cc.
April 18	74	99	2.6	167	51.5	3.21	1.60	1.65	54.9	27.4	28.2
May 2	50	96	6.1	329	44.5	3.69	2.05	1.64	63.1	35.1	28.1
May 11	50	96	6.5	256	42.5	3.93	2.26	1.67	67.2	38.7	28.6

In this case there was no anemia. The blood volume was low, entirely in respect of plasma, the cell volume being normal. With the subsidence of the edema the plasma volume rose to within normal limits and the cell volume underwent no change.

Case 2. L. E., number 16,501. Autopsy number 11030. Age 24 years. White male, previously an attendant in a gasoline filling station. Diagnosis: chronic nephritis.

Patient was admitted January 27, 1928, for massive edema involving most of the body, ascites and bilateral hydrothorax. In the three preceding years he had had three sudden attacks of swelling commencing in his legs and spreading up to his abdominal wall; the first two attacks lasted several months and had subsided suddenly and spontaneously; the third had persisted over a year up to the time of admission. There was no history of infection.

On admission, the red blood count was 3.7 million and the white count 13,000. The blood pressure was 176/110 mm. Hg. There were no retinal changes. The urine showed a massive albuminuria, a few granular and hyaline casts, and numerous doubly refractile crystals; there were no red cells and the specific gravity was 1030. The blood examination gave a nonprotein nitrogen of 31 mgm. per 100 cc.; serum protein of 4.2 grams per 100 cc. with inverted albumin-globulin ratio of 9.43 to 0.57; and a high cholesterol of 410 mgm. per 100 cc.

His weight on admission was 120 kgm. and on discharge 4 months later it had fallen to 78 kgm.

Four months later he was readmitted for a few days to repeat the blood volume and other determinations. Except for the loss of an additional 4 kgm. of weight and a progression in his anemia he was much the same as he had been 4 months previously. His red cell count had fallen to 2.5 million, his nonprotein nitrogen

TABLE 3
Variations in blood volume with disappearance of edema. Case 2

Date	Weight	Hemo- globin	Total protein	Choles- terol	Hema- tocrit	Volume			Volume per kgm. ideal weight		
						Whole blood	Plasma	Cells	Whole blood	Plasma	Cells
1928	kgm.	per cent	grams per 100 cc.	mgm. per 100 cc.	percent- age of cell volume	liters	liters	liters	cc.	cc.	cc.
January 31	116	62	4.2	410	34	3.99	2.63	1.35	55.5	36.4	18.9
March 2	109	63	3.5	306	31	5.16	3.56	1.60	71.7	49.5	22.2
April 13	88	62	3.8		29	5.14	3.45	1.69	71.5	48.0	23.5
May 21	80	60			31	4.89	3.37	1.52	67.9	46.9	21.1
October 20	74	45	4.6	313	25	5.28	3.96	1.32	73.4	55.0	18.4

was 45 mgm. per 100 cc. and his blood pressure 185/110 mm. Hg. There was still massive albuminuria, but in addition numerous casts and very occasional red cells were now present.

Eight months later he was readmitted in uremia and died.

In this case as shown in table 3 the blood volume was low, the plasma volume was on the lower limit of normal and the cell volume below the normal figure. There was a considerable anemia. After diuresis had set in the blood volume rose to the normal figure mainly as the result of a great increase in plasma volume which rose above the normal value. The cell volume also increased at first, but finally fell again with the progress of the anemia. In the last determination the total blood volume was still within normal limits despite the very

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low cell volume, because the plasma volume had risen well above the normal level.

Case 3. C. C. number 19,889. Age 27 years. White male, stationary engineer. Diagnosis: chronic nephritis with chronic sinusitis. Admitted August 5, 1928 for general anasarca.

Eight months earlier the swelling had started in the legs and had become generalized four weeks before admission.

On admission there was found to be generalized edema and a bilateral hydrothorax. The blood pressure was 122/72 mm. Hg. The red blood count was 4.7 million, and the white count 17,000. The urine contained great quantities of albumin, numerous casts, but no red cells. The nonprotein nitrogen was 70 mgm., cholesterol 500 mgm., total protein 3.3, grams per 100 cc., and there was an inverted albumin-globulin ratio of 0.30 to 0.70.

TABLE 4
Variations in blood volume as edema increased and diminished. Case 3

Date	Weight	Hemo- globin	Total protein	Choles- terol	Hema- tocrit	Volume			Volume per kgm. ideal weight		
						Whole blood	Plasma	Cells	Whole blood	Plasma	Cells
1928	kgm.	percent	grams per 100 cc.	mgm. per 100 cc.	percent- age of cell volume	liters	liters	liters	cc.	cc.	cc.
September 1	73	92	3.3		36.5	4.10	2.60	1.50	63.5	40.3	23.3
November 3	77	69	4.5	820	36.0	3.23	2.07	1.16	50.1	32.1	18.0
December 17	54	50	4.0	396	27.0	3.97	2.90	1.07	61.5	45.0	11.6

The patient's weight gradually increased during the first three months from 68 to 79 kgm. Acupuncture was then performed and repeated with a resulting reduction of weight to 52 kgm. in one month. He went home at Christmas for six weeks and then returned to the hospital for another three months study, during which time his weight varied between 66 and 58 mgm.

During the period in which he was under observation the blood pressure tended to rise and the anemia to increase, but the nonprotein nitrogen fell slightly. The urine underwent little change. At the time of his final discharge the blood pressure was 140/100 mm. Hg, and the red blood count was 3.0 million.

In this case (see table 4) on admission the whole blood and cell volume was low or near the lower limits of normal. The plasma volume was normal. With increasing edema the plasma volume fell markedly and the cell volume also fell with the progress of the anemia.

Later as the edema disappeared the blood volume approached again the lower limits of normal despite the still further diminishing cell volume, owing to the fact that the plasma volume had risen above the normal.

Case 4. N. M., number 16,638. Autopsy, number 10661. Age 47 years. German housewife. Diagnosis: chronic nephritis, arteriosclerosis, hypertension, myocardial insufficiency. Admitted February 3, 1928, for general anasarca.

The swelling of the legs had started two months before admission. More recently the abdomen had begun to swell, there had been puffiness of the face, misty vision, dyspnea on exertion and weakness.

There was found on examination generalized edema, ascites and a bilateral hydrothorax. The heart was large and there was marked albuminuric retinitis. The blood pressure varied between 200/120 and 220/160 mm. Hg. The urine

TABLE 5
Variations in blood volume with variations in amount of edema. Case 4

Date	Weight	Hemo- globin	Total protein	Choles- terol	Hema- tocrit	Volume			Volume per kgm. ideal weight		
						Whole blood	Plasma	Cells	Whole blood	Plasma	Cells
1928	kgm.	per cent	grams per 100 cc.	mgm. per 100 cc.	percent- age of cell volume	liters	liters	liters	cc.	cc.	cc.
February 17	68	85	4.1	248	40	3.70	2.22	1.48	59.2	38.5	23.7
March 3	60	75	5.0		32	4.63	3.15	1.48	74.1	50.4	23.7
May 17	69	80	4.2	186	32	4.12	2.80	1.32	65.9	44.8	21.1

contained large quantities of albumin, casts, and red cells. The red cell count was 4.1 million, the nonprotein nitrogen 42 mgm., total protein 4.7 grams per 100 cc. with inverted albumin-globulin ratio of 0.30 to 0.70. The cholesterol was 360 mgm. per 100 cc.

She was readmitted on a number of occasions owing to the return of the anasarca; and, although she improved temporarily each time, her progress was steadily down-hill. At the final admission, ten months after she was first seen, her nonprotein nitrogen had risen to over 100 mgm. per 100 cc. and she died in uremia.

On the first determination, when there was considerable edema, the blood volume was below normal. With the disappearance of the edema the plasma volume rose markedly to a value above normal, the cell volume remaining unchanged. On returning to the hospital the

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second time with more edema the plasma volume had fallen again, the cell volume being only slightly less than what it was previously. (See table 5.)

Case 5. A. S., number 26,662. Age 49 years, White male, "steamfitter." Diagnosis: chronic diffuse nephritis. Admitted September 3, 1929 for general anasarca.

The swelling had started gradually one year before admission. This patient was very uncomfortable and it was impossible to subject him to a blood volume determination except on one occasion. He reacted scarcely at all to the various forms of treatment instituted. There was considerable anemia present, the red cell count being around 3 million. The nonprotein nitrogen was 50 mgm., the cholesterol ranged between 165 and 250 mgm., the total protein was 3.8 grams

TABLE 6
Blood volumes in five cases of nephritis at time of maximum edema

Case	Age	Actual weight kgm.	Ideal weight kgm.	Serum protein grams per 100 cc.	Hemo-globin per cent	Actual volume			Volume per kgm. ideal weight		
						Whole blood	Plasma	Cells	Whole blood	Plasma	Cells
						liters	liters	liters	cc.	cc.	cc.
1	42	74	58.5	2.56	99	3.21	1.60	1.65	54.9	27.4	28.2
2	23	116.4	71.9	2.20	62	3.99	2.63	1.35	55.5	36.4	18.9
3	27	77	64.5	4.54	69	3.23	2.07	1.16	50.1	32.1	18.0
4	47	68	62.5	4.71	85	3.71	2.22	1.48	59.2	38.5	23.7
5	50	70	67.5	4.81	58	3.49	2.51	0.98	51.8	37.2	14.5

per 100 cc. and the albumin-globulin ratio was inverted, 41 to 59. The blood pressure was 140/85 mm. Hg. The urine showed enormous quantities of albumin, occasional casts, but no red cells.

The single blood volume determination on this patient showed a very low blood volume, 51.8 cc. per kilogram ideal weight; a very low cell volume, 14.5 cc. per kilogram ideal weight; and a normal plasma volume of 37.2 cc. per kilogram ideal weight.

DISCUSSION

The figures in these five cases of renal edema show that the total blood volume was markedly diminished at the time of greatest edema. In the first case in which there was no concomitant anemia the reduction was entirely in respect of plasma. In the remaining cases com-

plicated by varying grades of anemia the plasma volume was less definitely reduced, being either just below the normal limit or near the lower limit of normal. In these anemic cases the cell volume was also reduced, being most reduced in the cases with the greatest anemia. In the four cases on which more than a single determination was made the blood volume increased markedly, almost entirely in respect of plasma as the edema disappeared. In the anemic cases it seemed as though the plasma volume attempted to compensate for the diminished cell volume in order to bring the total blood volume to a normal level: in these cases therefore the plasma volume often rose to a figure much greater than that of the normal plasma volume.

It is clear from this that our results tend to be in agreement with the results of Darrow and of Plesch and generally opposed to those of Brown and Rowntree.

The figures we obtained indicate that the variations in the plasma volume are connected not so much with the amount of edema present in the individual patients as with the presence or absence of diuresis. Thus in cases 2, 3 and 4 the plasma volume had risen to normal or above normal long before the edema had disappeared. It would seem that when edema is *collecting* the plasma volume falls, and that while the edema is disappearing the plasma volume rises again. Hence it is possible that the edematous cases in Brown's and Rowntree's group had already begun to lose their edema, so that the initial stage of low plasma volume was missed.

Brown and Rowntree noted that cases of nephrosis *with anemia* tended to have blood volumes that were slightly above normal in respect of whole blood and of plasma. This is interesting in connection with our observation that in anemic edematous patients the plasma volume is not lowered to the same extent as in edematous patients without anemia, and that when diuresis has set in the plasma volume may rise above normal as if to compensate for the lowered cell volume and to bring the total blood volume up to the normal level.

Another factor that may explain some of the differences between our results and those of Brown and Rowntree is the difference in the two methods used. Brown and Rowntree used the dye method, whereas we used the carbon monoxide method. Personally we are of the opinion that there is considerable evidence that an appreciable part

of the dye diffuses into the lymph spaces and thus includes a portion of the lymph volume in the determined "blood volume." We have given our reasons for this view in two other papers now in press. If that view is accepted it may be quite possible that in certain cases of renal edema the tendency for the dye to diffuse is greater than it is in normal individuals. In that event one would expect the dye method to fail to show a diminution in the blood volume of patients suffering from renal edema. It could also explain why during diuresis the dye method sometimes yields a diminution rather than an increase in plasma volume.

SUMMARY

A study has been made of the blood volume in five cases of renal edema by means of the carbon monoxide method. The results obtained indicate that:

1. The blood volume is abnormally low when edema is at its height, that it increases as the edema disappears and may fall again if the edema subsequently reappears.
2. In a case uncomplicated by anemia the cell volume is normal and the changes are confined to the plasma volume.
3. When anemia is present there is a diminution in cell volume which is more marked the higher the degree of anemia. In anemic cases the plasma volume is less definitely reduced; and when diuresis sets in the plasma volume may rise above the normal as though to compensate for the diminished cell volume and to bring the total blood volume up to normal.

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EFFECT OF ACIDOSIS AND ALKALOSIS UPON CAPACITY FOR WORK

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The composition of arterial blood in exercise varies with the intensity of the exertion. In moderate work, percentage saturation of hemoglobin, alkaline reserve and hydrogen ion concentration remain constant or nearly so while the proportion of hemoglobin in blood and of protein in serum increase from 5 to 10 per cent. When exercise is carried on to exhaustion there is little or no further increase in concentration of blood proteins but lactic acid concentration may increase to 11 m.Eq. per liter and carbonic acid capacity² may decrease in nearly reciprocal fashion. There may be a decrease in arterial pH, of 0.3 which means a liberation of base bound by protein. Work cannot be continued if lactic acid concentration much exceeds 10 m.Eq. per liter of blood.

In circulatory diseases the description cannot be given in such precise fashion. The capacity for oxygen transport to the tissues is diminished, the composition of the blood in rest is variable, and it has been suggested by Eppinger and his associates and by Pilcher, Clark and Harrison (1930) that the buffering power of the blood and tissues is diminished in congestive heart failure.

In view of the complications associated with exercising a sick man we have established acidosis in a normal man (H. D.) by ammonium chloride administration and have studied his performance while normal while in acidosis and while in the alkalosis subsequent to withdrawing ammonium chloride. The composition of urine and the respiratory changes in rest have been described by Dennig, Dill and Talbott (1929).

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² The expression *carbonic acid capacity* denotes the carbonic acid content of oxygenated blood equilibrated at 37.5° with CO₂ at a partial pressure of 40 mm. This quantity is also referred to as T₄₀.

EFFECTS OF ACIDOSIS AND ALKALOSIS

TABLE 1
Observations on equilibrated blood

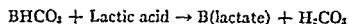
Experi- ment number	Date	Condition	HbO ₂ capacity	pCO ₂	Total CO ₂		Calcu- lated
			<i>m.Eq. per liter</i>	<i>mm.Hg.</i>	<i>m.Eq. per liter blood</i>	<i>m.Eq. per liter serum</i>	pH _s
1	May 28	Acidosis, Rest	9.11	41.6	17.9	20.7	7.28
				74.8	22.5	25.7	7.11
		Work	9.64	30.8	11.5	13.6	7.22
				68.0	17.4	19.8*	7.02
2	June 6	Alkalosis, Rest	7.76	43.6	25.9	31.2	7.45
				74.9	31.5	37.2*	7.28
		Work	8.70	43.2	23.2	28.4	7.41
				74.4	28.4	34.0*	7.24
3	June 22	Normal, Rest	9.02	36.3	19.9	24.5	7.42
				72.2	26.4	31.4*	7.22
		Work	9.56	33.9	17.8	21.8	7.40
				70.1	24.7	29.1*	7.20
4	September 27	Normal, Rest	9.06	46.7	22.6	26.5	7.34
				79.2	27.5	31.7*	7.18
		Work	10.50	39.4	16.6	20.2	7.29
				98.2	25.3	29.4*	7.04
5	September 29	Acidosis, Rest	10.05	26.9	9.68	11.3	7.20
				59.9	15.1	17.1*	7.02
		Work	10.80	31.1	8.74	9.84	7.07
				68.8	14.2	15.5*	6.90
6	October 1	Alkalosis, Rest	7.86	29.6	22.5	27.9	7.57
				64.5	29.7	35.6*	7.33
		Work	8.75	34.4	20.1	24.2	7.44
				70.2	26.7	31.2*	7.23

* The indicated values were not determined experimentally but were calculated from the line charts developed by Van Slyke and Sendroy (1928).

The essential observations on oxygenated blood equilibrated with carbon dioxide are given in chronological order in table 1. It will be noted that two sets of experiments were carried out with an intervening period of about three months. The details with regard to diet and ammonium chloride intake in the former set of experiments will be found in the paper referred to above; in the second set of experiments the same diet was followed and 15 grams of ammonium chloride were taken on two days only, September 27 and 28. After the acidosis experiment on the 29th and again on the 30th, 10 grams of sodium bicarbonate were taken. The resulting alkalosis was so severe that slight tetanic cramps were experienced the night of September 30-October 1. These had disappeared by the time the alkalosis experiment was begun on October 1.

The exercise in the first set of experiments consisted in running at the rate of 7.4 km. per hour for 15 minutes. The subject had had little experience on the treadmill at this time and his performances were not skillful. During September he had 12 practice runs at 9.3 km. per hour and succeeded finally in running at this speed with a fairly constant day-to-day performance and with about the same oxygen consumption as at the slower rate. On the basis of these experiments, but particularly on the last set with the subject in better training and running at a faster speed, it is possible to make an estimate of the effects of acidosis and of alkalosis upon the performance of a given task.

The lactic acid which accumulates during exhausting work can be neutralized by three mechanisms working together.³ The simplest is by the reaction



In order that this mechanism may function it is necessary for the lung to put out carbonic acid faster than it is produced by oxidative processes, and this in fact is what occurs, the result being an apparent increase in respiratory quotient. It is physiologically impossible to increase alveolar ventilation enough in the extreme acidosis of exhaus-

³The possible buffering effect of phosphocreatine has been neglected in the absence of evidence that hydrolysis of this substance is quantitatively important in normal muscular activity.

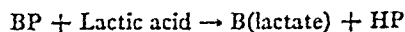
tion to maintain the ratio $\frac{H_2CO_3}{BHCO_3}$ at its resting level and the result is that, although the reaction of arterial blood in moderate work may be the same as during rest, in exhaustion there is always an increase

TABLE 2
Buffer value calculations

Experiment number	Condition	$\Delta BHCO_3$ <i>m.Eq. per liter blood</i>	Protein <i>grams per liter blood</i>	$\frac{-\Delta BHCO_3 \times 10^4}{(\Delta pH) (\text{protein})}$	T_{40} * <i>m.Eq. per liter blood</i>
1	Acidosis,				
	Rest	3.6	199	106	17.6
	Work	4.8	207	116	13.1
2	Alkalosis,				
	Rest	4.7	180	153	25.2
	Work	4.3	193	131	22.5
3	Normal,				
	Rest	5.5	198	139	20.7
	Work	5.9	206	143	19.1
4	Normal,				
	Rest	4.0	198	126	21.5
	Work	7.0	220	127	16.6
5	Acidosis,				
	Rest	4.5	213	117	12.1
	Work	4.4	224	115	10.2
6	Alkalosis,				
	Rest	6.1	181	140	25.2
	Work	5.6	194	137	21.2

* The expression *carbonic acid capacity* denotes the carbonic acid content of oxygenated blood equilibrated at 37.5° with CO₂ at a partial pressure of 40 mm. This quantity is also referred to as T_{40} .

in acidity. This brings into action the third mechanism, by means of which base bound by protein is set free to neutralize lactic acid:



To what extent are these mechanisms modified by the conditions in our experiments? Assuming that parallel changes take place in

blood and in muscles insofar as the principal anions are concerned, it is possible to estimate the effect of variation in carbonic acid capacity upon the buffer value of the protein-bicarbonate system. For this

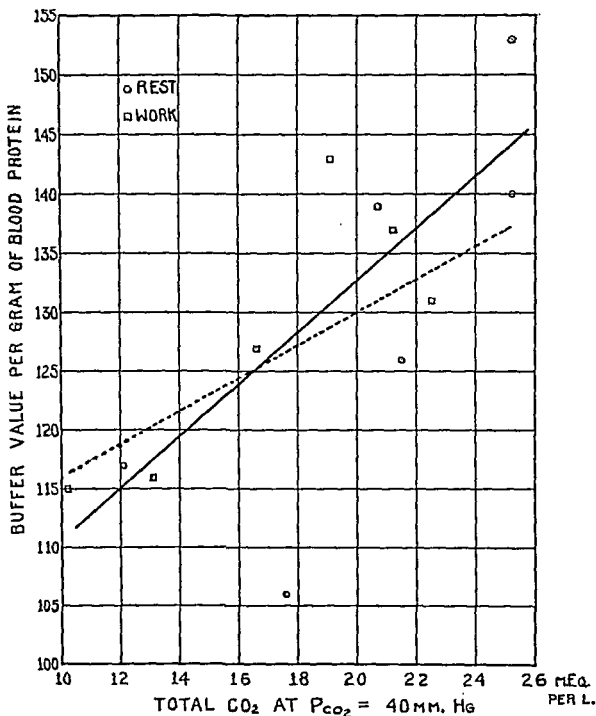


FIG. 1

purpose the calculations shown in table 2 have been made. Column 3 has been derived in an obvious manner from the observations on equilibrated blood given in the preceding table and from the solubility determinations for carbon dioxide in serum and cells made by Van

Slyke, Sendroy, Hastings and Neill (1928). A measure of buffer value of blood as defined by Van Slyke may be obtained by dividing ΔBHCO_3 by values for ΔpH , derived from table 1. These buffer values are related in a linear fashion to blood protein concentration and in order to eliminate this variable the buffer values per unit of blood protein have been calculated as appears in column 5.⁴

It is now possible to determine the relation between buffer value and carbonic acid capacity. The last column in this table gives the total carbonic acid content of each specimen of blood at a carbonic acid pressure of 40 mm. Figure 1 illustrates the relation between the variables *total carbonic acid at $p\text{CO}_2 = 40$ mm.* and *buffer value per unit of blood protein.* The points show considerable fluctuation partly due, no doubt, to experimental errors and perhaps also to failure to take into account other variables. (Thus comparison is made over the physiological range but not over the same range in pH .)

An equation showing the straight line trend of these points has been derived according to the Pearson formula,

$$y = Rx - Rx_m + y_m$$

in which x_m = mean value of x

y_m = mean value of y

$$R = \frac{n(\Sigma xy) - \Sigma x \Sigma y}{n \Sigma x^2 - (\Sigma x)^2}$$

n = number of observations

If y = buffer value and x = carbonic acid capacity, one derives an equation in which the experimental errors are assumed to lie principally in the determination of y and of course this assumption is correct, for x can be determined precisely. The resulting relationship is

$$\text{Buffer value} = 2.1 (\text{carbonic acid capacity}) + 89$$

The solid line shown on figure 1 corresponds to this equation. The fact that the regression coefficient of this equation is 2.1 ± 0.57 indicates that the slope is only roughly defined.

⁴ There is an error involved in this calculation in the assumption that serum protein and hemoglobin have the same buffer value per unit weight but for our present purposes this error is negligible.

It is interesting to see to what extent these results are in accord with the empirical description of carbonic acid dissociation curves given by Henderson and associates (1930). To make such a comparison we have prepared table 3. This shows for each experiment the observed ΔCO_2 (60-30) and the values for the same variable as estimated from figure 3 of the paper just referred to. The last column is derived as indicated and is a measure of the change in buffer value of blood proteins with T_{10} as implied by this empirical chart. The mean value of R is 1.00 but the positive and negative errors are so distributed

TABLE 3
Buffer values from the empirical description of CO_2 curves (Henderson et al.)

T_{10}	$\Delta\text{CO}_2(60-30)$			$\frac{-\Delta\text{BHCO}_2 \times 10^3}{(\Delta\text{pH}) (\text{protein})} \times \frac{1}{R}$
	Experimental	Calculated	$R = \frac{\text{Experimental}}{\text{Calculated}}$	
<i>m.Eq. per liter</i>	<i>m.Eq. per liter</i>	<i>m.Eq. per liter</i>		
17.6	4.9	5.50	0.89	119
13.1	5.0	5.09	0.98	118
25.2	6.5	5.96	1.09	141
22.5	6.0	6.00	1.00	131
20.7	6.1	5.87	1.04	134
19.1	6.1	5.87	1.04	137
21.5	5.7	6.00	0.95	133
16.6	5.5	5.82	0.95	134
12.1	4.7	4.90	0.96	122
10.2	4.4	4.57	0.99	116
25.2	6.4	6.00	1.07	131
21.2	6.0	5.83	1.03	133

that when one calculates the trend of these points as before the equation comes out

$$\text{Buffer value} = 1.4 (\text{carbonic acid capacity}) + 102$$

The broken line in the figure corresponds to this equation. It appears, from our experimental observations, that when the carbonic acid capacity is reduced by one-half the buffer value of blood proteins is reduced by one-sixth. On the basis of the Henderson chart the reduction is one-ninth.

The empirical chart just referred to is based wholly on experimental

observations on blood in this laboratory. A similar chart was prepared from previous knowledge of the properties of the components of the system (fig. 5 in the same paper). One of the assumptions was expressed by the relation developed by Van Slyke

$$\text{BHbO}_2 = 3.6 \text{ Hb } (\text{pH}_e - 6.41)$$

We find that it is now necessary in order to express the facts more accurately to use the equation

$$\text{BHbO}_2 = \text{Hb } (9.75 \text{ pH}_e - 0.5 (\text{pH}_e)^2 + n)$$

While there must remain some doubt regarding the magnitude of this effect, its reality is obvious. It was in fact suggested by Dill, Bock, Lawrence, Talbott and Henderson (1929). Figure 8 in that paper shows blood bicarbonate as a function of pH_e in various bloods. No account was taken of variation in protein concentration and hence the slopes of the different curves are variable on that account as well as on account of variation in carbonic acid capacity. However, there is no question about the change in slope with pH_e in diabetic coma. A tangent is drawn to the curve of T. F. B. in the physiological range. Its slope is very different from that in normal blood. The buffer value calculated as above, taking into account blood protein concentration, comes out as 30, one-fourth of the normal value.

It was pointed out in that paper that such a result should not have been wholly unexpected. Thus if one extrapolates the straight line corresponding to horse blood, as determined by Van Slyke, it intersects the line $(\text{BHCO}_3)_b = 0$ at a pH_e value of 7.94. This does not happen in normal blood, indicating again that as one reaches low values of $(\text{BHCO}_3)_b$ buffer value decreases.

There remains the question of hydrogen ion concentration in its relation to breathing and the liberation of base from protein. The observations on oxygen consumption and on ventilation are given in table 4. Samples of alveolar air were obtained and from the carbon dioxide pressure in these samples and the carbonic acid dissociation curves described in table 1 it was possible to derive the values for pH of arterial serum and for total CO_2 content of arterial blood which are shown in table 4. The same table contains the values for lactate content of blood.

The values for oxygen consumption in work given in this table show greater variation from day to day than that observed in an athlete performing a fixed task. This may be in part related to the physical condition of the runner. On certain days, particularly on May 28, September 29, and October 1, the subject was in some distress before work began and hence ran with less perfect nervous coordination than

TABLE 4
Respiratory changes and composition of arterial blood

	Experi- ment 1	Experi- ment 2	Experi- ment 3	Experi- ment 4	Experi- ment 5	Experi- ment 6
	Acido- sis	Alka- losis	Nor- mal	Nor- mal	Acido- sis	Alka- losis
Oxygen used, liters per minute:						
Work.....	2.69	1.96	2.19	2.36	2.75	2.52
Ventilation, liters per minute:						
Rest.....	6.4	4.8	5.4	5.4	8.5	5.6
Work.....	82	46	54	48	80	51
Work ÷ Rest.....	13	10	10	9	9	9
pH _a :						
Rest.....	7.38	7.46	7.39	7.41	7.21	7.47
Work.....	7.25	7.43	7.38	7.30	7.09	7.39
Δ.....	-0.13	-0.03	-0.01	-0.11	-0.12	-0.08
(Total CO ₂) _a , m.Eq. per liter:						
Rest.....	15.6	25.7	20.6	21.2	9.6	25.2
Work.....	10.8	23.0	18.2	16.5	8.1	21.3
Δ.....	-4.8	-2.7	-2.4	-4.7	-1.5	-3.9
(Lactate) _a , m.Eq. per liter:						
Rest.....	0.9	0.8	0.9	1.3	0.7	1.4
Work.....	4.6	3.2	2.5	5.0	2.7	4.5
Δ.....	+3.7	+2.4	+1.6	+3.7	+2.0	+3.1

at other times. Some such explanation is more probable than one involving difference in intrinsic processes.

The limiting value for total ventilation in non-athletic subjects usually lies between 75 and 100 liters per minute and it is probable that the subject had reached his maximum rate on May 28 and September 29. The alveolar carbon dioxide pressure in work on September 29

was 28 mm. and this probably can be taken as near the physiological limit for, in this case, the pH of arterial serum was 7.09 and that of venous serum must have been about 6.95. These reactions are even more acid than those usually found in diabetic coma.

TABLE 5
Limiting values for T_{10} and arterial pH, in normal men

Subject	Exercise	Lactic acid	T_{10}	pH of arterial serum*
		<i>m.Eq. per liter</i>	<i>m.Eq. per liter</i>	
J. W.....	Rowing	9.8	12.1	7.11
J. R.....	Rowing	11.0	10.4	7.02
M. H.....	Running	9.6	13.5	7.16
J. H. T.....	Running	8.4	12.7	7.14 ± 0.04
A. V. B.....	Running	9.4	11.7	7.08 ± 0.04
D. B. D.....	Running	10.5	13.2	7.14

* The values for pH of arterial serum were calculated from the dissociation curve of true plasma of oxygenated blood and from the carbon dioxide pressure of samples of alveolar air, assuming that such samples represent air in complete equilibrium with arterial blood.

TABLE 6
Effect of acidosis and alkalosis on capacity of blood for neutralizing lactic acid

	Experiment 1	Experiment 2	Experiment 3	Experiment 4	Experiment 5	Experiment 6
	Acidosis	Alkalosis	Normal	Normal	Acidosis	Alkalosis
Total CO_2 , arterial blood in rest, <i>m.Eq. per liter</i>	15.6	25.7	20.6	21.2	9.6	25.2
Maximum ΔBHCO_3 of arterial blood from rest to exhaustion, <i>m.Eq. per liter</i>	-7.5	-17.6	-12.5	-13.1	-1.5	-17.1
pH of arterial serum, rest.....	7.38	7.46	7.39	7.41	7.21	7.47
Maximum ΔpH of arterial serum, rest to exhaustion.....	-0.29	-0.37	-0.30	-0.32	-0.12	-0.38
ΔBP due to such a change in pH, <i>m.Eq. per liter</i>	-7.1	-9.1	-7.6	-8.7	-3.0	-9.4
Acid neutralizing capacity, - ($\Delta\text{BHCO}_3 + \Delta\text{BP}$), <i>m.Eq. per liter</i>	+14.6	+26.7	+20.1	+21.8	+4.5	+26.5

In various unpublished observations we have indirect evidence that the reaction reached in this experiment is near the limit for man starting from a normal state. Table 5 is a summary of six experiments in which men started from a normal state and pushed themselves to

exhaustion with lactic acid concentrations of 9 to 11 m.Eq. per liter. Two subjects were oarsmen in good training and there was not a striking difference between the composition of their blood in exhaustion and that of the four untrained subjects. No experiments with a subject starting from a state of alkalosis have been carried out aside from the two on H. D. However, since the limiting values for pH and carbonic acid capacity reached during exhausting work are of the same order of magnitude whether one starts from a normal state or from a state of acidosis, it appears probable that the same is true starting from a state of alkalosis. We shall make this assumption tentatively and proceed to calculate the amount of acid which could have been neutralized per liter of blood between the observed values of pH_a and of carbonic acid content in rest and the assumed limiting values in exhaustion, viz., pH_a = 7.09 and total carbonic acid content of arterial blood = 8.1 m.Eq. per liter. The calculations by which table 6 is derived are made by a simple application of the data in the figure (solid line) and in tables 1, 2 and 4. The calculation in the first experiment may be shown for illustration:

Total CO ₂ of arterial blood in rest, <i>m.Eq. per liter</i>	15.6
Minimum CO ₂ of arterial blood in exhaustion, <i>m.Eq. per liter</i>	8.1
Δ Total CO ₂ of arterial blood rest to exhaustion, <i>m.Eq. per liter</i>	-7.5
pH of arterial serum, rest.....	7.38
Minimum pH of arterial serum, exhaustion.....	7.09
ΔpH, rest to work.....	-0.29
Blood protein in work, <i>grams per liter</i>	207
Carbonic acid capacity in rest, <i>m.Eq. per liter</i>	17.6
Carbonic acid capacity in exhaustion, <i>m.Eq. per liter</i>	10.2
Mean carbonic acid capacity in work, <i>m.Eq. per liter</i>	13.9
Corresponding protein buffer value.....	0.119
ΔBP = (Buffer value) (ΔpH _a) (Protein), <i>m.Eq. per liter</i>	-7.1
Acid neutralizing capacity = -(ΔBHCO ₃ + ΔBP), <i>m.Eq. per liter</i> ...	14.6

It is necessary to emphasize that the neutralizing capacities shown in table 6 are for blood and that it is probable that the capacity of tissues, per unit weight, for neutralizing acid is less than that of blood. Thus the results of Fenn (1928) indicate that muscle has a flatter dissociation curve and a lower carbonic acid capacity than blood. However, it is quite probable that acidosis and alkalosis have parallel

effects on blood and on tissues. Taking the maximum neutralizing capacity of normal blood in rest as 100, the other values are as follows:

<i>Acidosis</i>		<i>Alkalosis</i>	
June 6 (nearly compensated).....	70	June 22.....	127
September 29.....	21	October 1.....	126

There is conclusive evidence that the capacity for oxygen debt is decreased when work is undertaken in ammonium chloride acidosis. On September 29, the subject was unable to run longer than 15 minutes although his lactic acid concentration was only 2 m.Eq. per liter of blood. In other experiments he ran for 18 or 20 minutes, sometimes with much more lactic acid, and yet with less distress at the finish.

It appears from table 6 that a runner starting off in alkalosis can pile up a greater oxygen debt than when in his normal state. The work was not severe enough to give this hypothesis a rigid test but the problem has interesting possibilities and may be investigated further at a later time.

There is an ambiguity of considerable importance which awaits further investigation. The observed increases in lactic acid in Dennig's second acidosis experiment and in the six experiments summarized in table 5 are very much less than the estimated acid neutralizing capacities. There are no complete observations on the acid-base equilibrium in such a state of exhaustion and it would be unprofitable to seek for an explanation without such information.

There are obvious connections between these results and those obtained in heart disease. Clearly the capacity to accumulate an oxygen debt decreases with carbonic acid capacity because the amount of bicarbonate available for neutralizing lactic acid is decreased and at the same time the buffer value of proteins decreases with decrease in carbonic acid capacity. In heart disease there may be some additional modification of muscle proteins in respect to their buffer value as suggested by Pilcher, Clark and Harrison, but our studies contribute nothing to this question. They have found evidence of some such change only when edema is present and it is possible that the phenomena they have observed are related in some way to modification of diffusion processes in the tissues by increase in intracellular fluid.

SUMMARY

A study has been made of the effects of muscular activity on properties of the blood in three states: (1) normal; (2) acidotic due to ammonium chloride intake and (3) in a state of alkalosis subsequent to ammonium chloride intake.

The buffer value of blood proteins is reduced one-ninth or more with a decrease of one-half in carbonic acid capacity. Due to this effect as well as to decrease in carbonic acid capacity, the ability to neutralize lactic acid and hence to accumulate an oxygen debt is greatly curtailed when one begins exercise in a state of acidosis. Inferentially, the ability to accumulate an oxygen debt is increased by establishing a state of alkalosis before beginning exercise.

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STUDIES OF SERUM ELECTROLYTES

VII. THE TOTAL BASE AND PROTEIN COMPONENTS OF THE SERUM DURING LOBAR PNEUMONIA WITH A NOTE ON THE GASTRIC SECRETION

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In previous papers (1) (2) (3), the variations in the composition of blood serum and some features of the metabolism of electrolytes during the course of lobar pneumonia have been described. Among the changes in the serum it was found that during the precritical period there is a decrease in total base concentration and a tendency to a decrease in protein concentration. This investigation has here been extended to include measurement of the separate components of the total base and protein of the serum.

There is comparatively little available data as to the concentration of sodium, potassium, calcium, and magnesium in the serum during pneumonia. This is to be expected because of the lack of appropriate methods, since most of the earlier methods required large amounts of blood. Peabody (4) reviewed the early literature and studied the concentration of calcium and magnesium in the serum during the course of lobar pneumonia. He found that both tended to be slightly lower during the febrile period than in convalescence. The average of 6 magnesium analyses during the febrile period was 19 per cent lower than the average of 4 analyses made after the crisis. The average of 7 measurements of calcium concentration during the febrile period was 10 per cent lower than the average of 4 measurements made after the crisis.

Gerstenberger, Burhans, Smith, and Wetzel (5) studied the con-

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centration of calcium and inorganic phosphorus in the blood of children suffering from pneumonia and found a reduction in both. Jansen (6) concluded that the calcium concentration is lowered during the precritical period and increased in the epicritical.

There is a large literature on the excretion of the various components of base in the urine during various fevers. The early literature was extensively reviewed by Garratt (7). The essence of the findings is that there is a decreased urinary excretion of sodium and calcium and an increased excretion of potassium during the height of the fevers, not infrequently continuing after the fall in temperature. Peabody observed these changes in pneumonia and also found magnesium to be excreted normally or in excess. Recently (8) we have reported a lowered excretion of total fixed base in the urine during the precritical period associated with the lowered excretion of chloride.

Attempts to explain the alteration in the concentration of the electrolytes during pneumonia have been made repeatedly but with doubtful success. In a recent contribution Binger, Christie, Davis, and Hiller (9) attacked the problem by measuring the serum chlorides in dogs, first, during experimental pneumococcus infection; second, during anoxemia; third, after tissue destruction; fourth, in anaphylactic shock; fifth, in experimental leucocytosis; and sixth, during fever. A significant decrease in serum chloride was found in all three animals infected by bronchial insufflation with the pneumococcus but in none of the other conditions studied. This study excludes from the entire complex of mutually dependent processes in a pneumococcus infection many of the individual factors which might be considered in seeking an explanation for the electrolyte disturbance.

Geill (10) has recently reviewed the literature on the albumin and globulin concentrations in the serum under normal and pathological conditions. His review brings out the fact that globulin is increased at the expense of albumin in most infections. Limbeck and Pick (11) have shown that in acute infections an increase in globulin may occur even when there is a decrease in total nitrogen.

In a series of five cases of pneumonia studied before and after the crisis Loeper, Ravier, and Lebert (12) have shown in each case a rise in globulin after the crisis. There was a 4 to 37 per cent increase in the ratio of globulin to total nitrogen in the serum after the crisis as compared with the serum before the crisis.

Horvath and Little (13) measured the nitrogen fractions of the serum taken from cows suffering with bronchopneumonia. During the course of the disease they found a rise in the globulin, fibrin, and nonprotein nitrogen.

METHODS

Inasmuch as we were interested in the changes coincident with the ordinary course of the disease, the patients studied were given no unusual treatment. They were on an average caloric intake of 800 calories per day during the febrile period. It is our experience that except when beverages containing glucose have been added to the diet this is the largest daily caloric intake tolerated as a rule by the patient acutely ill with pneumonia without the development of tympanites.

The blood from the patients studied was obtained in the morning before breakfast and was withdrawn by venipuncture into 50 cc. pyrex centrifuge tubes. The blood was collected under paraffin oil, out of contact with air, and was immediately centrifuged at high speed. The clear serum was removed under oil and was used for analysis. The serum was separated within twenty minutes after the blood was withdrawn. Brems (14) believes that high potassium values are found when serum is not separated immediately from the cells, due to the diffusion of potassium from the red blood cells. For this reason we have taken precaution to separate the serum at once.

Total base, sodium, and potassium analyses were made on ashed sera. The ashing was carried out by digesting the serum in silica beakers on an electric sand bath using 0.5 cc. concentrated H_2SO_4 and 1 cc. concentrated HNO_3 to each cc. of serum. The heat of the sand bath was raised gradually over a period of twenty-four hours and the contents in the beaker were evaporated to dryness. The beakers were then transferred to an electric furnace and the contents were ashed to a dark cherry heat (approximately $600^\circ C.$) for twenty minutes. When cool, one drop of 4 N H_2SO_4 was added and the material was heated again in the furnace to dark cherry heat for twenty minutes. With this method of ashing no excess of H_2SO_4 was present and the ash dissolved readily in water.

Sodium. The procedure recommended by Barber and Kolthoff (15) was employed in which sodium is measured gravimetrically as a precipitate of uranyl zinc sodium acetate, $(UO_2)_2ZnNa(CH_3COO)_9 \cdot 6H_2O$. Analyses were made on 1 cc. of serum. Although phosphates in large amounts affect the final result, in amounts of 12 mgm. per 100 cc., theoretical results were obtained. The precipitation and washing were carried out at the same temperature to avoid change in solubility coefficient.

Potassium was measured by the titrimetric method of Shohl and Bennett (16) using 2 cc. amounts of serum. Known solutions of potassium were analyzed with each series as a check. The values obtained on known solutions were always high by 0.2 to 0.6 mM. and the corresponding correction was subtracted from the results obtained on the unknown.

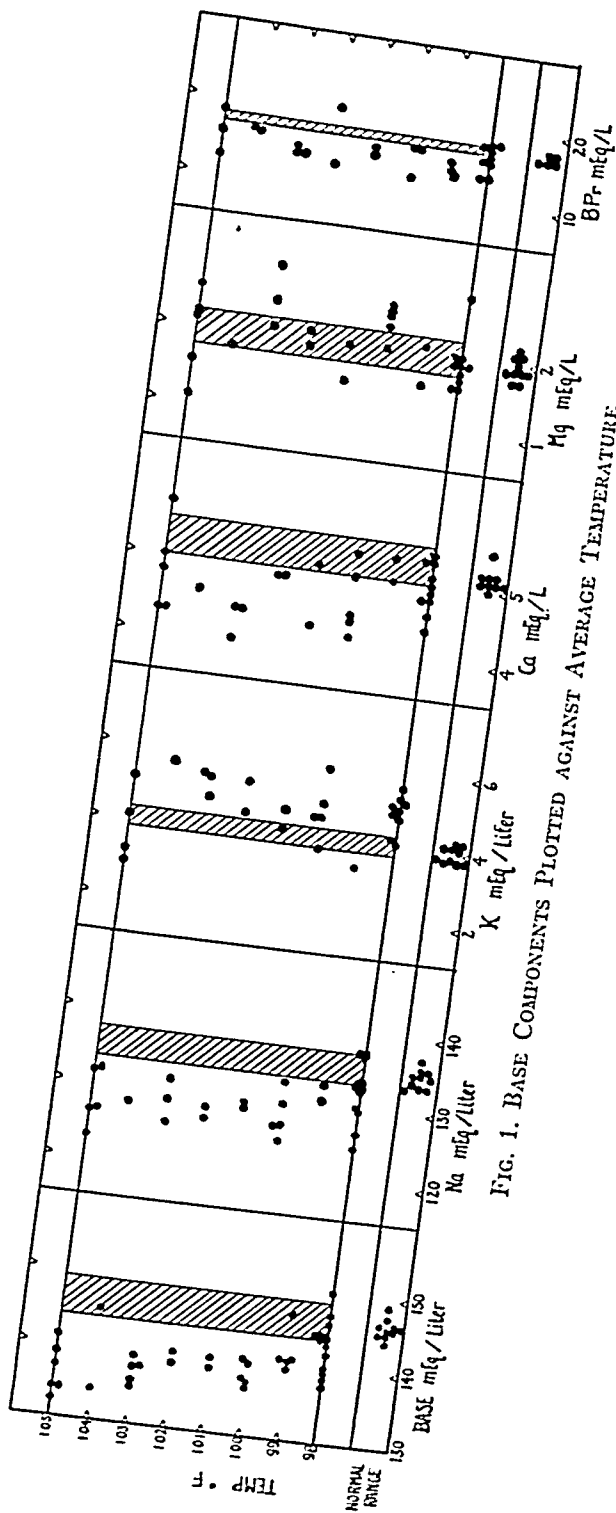


FIG. 1. BASE COMPONENTS PLOTTED AGAINST AVERAGE TEMPERATURE

Calcium was measured by the method recommended by Clark and Collip (17). *Magnesium* was measured in serum from which the calcium had been precipitated. The procedure was carried out by Briggs' method (18) in which magnesium is precipitated as $MgNH_4PO_4 \cdot 6H_2O$. The phosphorus content was then determined according to the colorimetric method of Fiske and Subbarow (19). *Total base* was analyzed according to the method of Stodie and Ross (20). *Albumin and globulin* measurements were made by the method of Howe (21) with a macro-Kjeldahl technique. *Base bound by protein*. This value was calculated by the equation of Van Slyke, Hastings, Hiller, and Sendroy (22). $BPr = 0.78 (\text{Albumin N}) (\text{pH} - 5.16) + 0.48 (\text{globulin N}) (\text{pH} - 4.89)$. A constant pH of 7.35 was assumed.

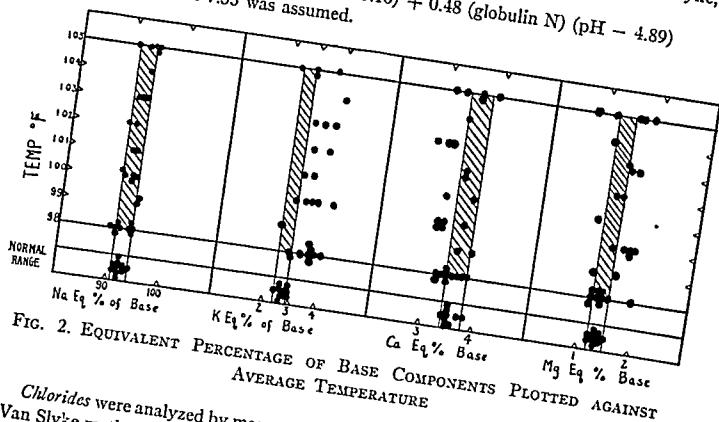


FIG. 2. EQUIVALENT PERCENTAGE OF BASE COMPONENTS PLOTTED AGAINST AVERAGE TEMPERATURE

Chlorides were analyzed by means of the Wilson and Ball modification (23) of the Van Slyke method. CO_2 analyses were made by the Van Slyke and Neill method (24). The Fiske and Subbarow (19) method of measuring inorganic phosphates was employed.

RESULTS

We are presenting our data of our base fractionations by plotting each type of base against the average temperature of the day on which the blood was withdrawn. It is often difficult to estimate correctly the day of crisis or even the day of the disease. For this reason we preferred to consider our findings in relation to the temperature (figure 1). Such an arrangement at least dissociates the febrile from the afebrile periods.

SERUM ELECTROLYTES IN PNEUMONIA

TABLE 1
Individual case analyses

Case number	Date	Temperature °F.	Serum Na m.Eq. per liter	Serum K m.Eq. per liter	Serum Ca m.Eq. per liter	Serum Mg m.Eq. per liter	Serum total base *			Serum protein N grams per liter	Albumin N grams per liter	Globulin N grams per liter	Nonprotein N grams per liter	Calculated BPr m.Eq. per liter	Serum Cl m.Eq. per liter	Serum CO ₂ mM. per liter	Serum P (inorganic) mgm. per 100 cc.
							Sum of cations m.Eq. per liter	Stadie and Ross method m.Eq. per liter	Average m.Eq. per liter								
C1	February 14 1930	101	128.7	4.2	5.3	2.0	139.4	140.0	139.7	10.00	5.30	4.70	0.30	14.8	99.6		
	February 24	99	132.8		5.0	1.6		140.6									
C2	February 15	105	131.5	3.2	5.7	1.1	141.2	137.0	139.1								
	February 24	98	124.8	5.6	4.8	1.6	137.1	139.5	138.8								
C3	March 17	104	127.9	5.6	4.6	1.8	139.7	134.3	137.0								
	March 20	99	130.7	3.4	5.3	2.1	141.5	137.6	139.6				0.24				
	March 24	98	132.9	5.0	5.3	1.8	145.0	138.6	141.8				0.28				
	March 31	98	133.8	4.8	5.1	1.9	145.6	150.4	148.0				0.33				
	April 10	98		5.3	2.0	2.0	147.2						0.40				
C4	March 3	105	123.0	2.9	4.3	2.2	132.4	132.4	132.4	11.47	5.50	5.96	0.33	15.6	89.7		
	March 6	98	126.6	4.9	4.4	2.0	137.9	137.6	137.8	11.94	7.77	4.17	0.38	18.2	97.8	4.2	
C5	March 10	100	124.9	4.6	4.4	2.4	136.4	137.3	136.9								
C6	March 10	103	125.6	4.8	4.0	2.9	138.6	135.1	136.9								
	March 17	100	129.7	5.9	4.3	2.0	141.9	137.8	139.9								
C7	March 25	103	131.4	4.3	4.4	2.2	142.3	139.3	140.8				0.26				
	March 31	98	129.7	5.3	4.8	1.9	141.7	143.3	142.5	10.07	5.62	4.45	0.28	90.2			
	April 14	98	137.6	4.1	5.0	2.8	149.5	144.1	146.8	11.88	6.50	0.28	14.8	88.9			
													17.5	98.0			2.3

C8	March 27	105	131.8	4.1	5.0	1.6	142.5	138.8	140.7	12.96	6.55	6.41	0.27	18.4			
	March 31	102	128.3	4.5	4.9	1.9	139.6	138.9	139.3								
	April 3	102	126.8	5.3	4.9	2.1	139.0	140.4	139.7	13.13	7.38	6.75	0.32	20.4			
	April 7	100	127.0	5.0	5.0	2.6	140.5	139.9	140.2	11.38	5.72	5.66	0.31	16.5			
	April 10	101	129.4	4.7	5.1	1.5	140.7	138.8	139.3	10.86	5.70	5.16	0.29	15.8			
	April 23	98	130.2	4.2	5.4	1.6	141.4	144.9	143.2	12.68	6.17	6.51	0.26	18.2	97.4		
C9	April 3	103	128.7	5.4	4.4	2.1	140.6	137.8	139.2	9.76	5.49	4.26	0.35	14.1	91.8		2.1
	April 7	100	132.6	4.6	4.5	2.5	144.2	140.4	142.3	11.35	5.60	5.75	0.32	16.4	93.1		
	April 14	98	137.5	5.1	4.9	1.7	149.2	144.6	146.9	11.48	5.33	6.15	0.25	16.4			4.4
	April 23	98	133.2	5.2	4.6	1.9	144.9	140.2	142.6	12.55	6.12	6.43	0.28	18.0	98.0		
C10	April 7	100	126.8	3.8	4.2	2.3	137.1	136.6	136.9	8.87	4.22	4.65	0.41	12.7	89.6		4.2
C11	April 8	105	126.3		4.8	2.1		134.1		10.03	6.92	3.11	0.31	15.5			
C12	April 22	105	126.6	5.1	4.3	2.6	138.6	141.0	139.8								
	1929																
C13	February 11	104								10.63	6.47	4.16	0.42	16.0	98.0		26
	February 14	104						145.0		11.12	6.23	4.89	0.50	16.4	101.0		28
	February 18	99						141.0		10.48	5.63	4.85	0.42	15.4	99.0		27
	March 4	98						146.0		10.85	5.77	5.08	0.20	16.0	99.0		31
C14	February 13	105						134.0		8.09	5.62	2.47	0.32	12.5	97.0		22
	February 16	102								8.27	6.09	2.18	0.49	13.0	95.0		24
	February 20	99						147.0		9.75	5.02	4.69	0.42	14.1	96.0		
	February 28	98						144.0		9.36	5.18	4.18	0.27	13.8	97.0		32
C15	March 10	103						136.0		9.28	5.51	3.77	0.49	13.9	86.0		27
	March 13	99						141.0		9.04	5.58	3.46	0.52	13.6	91.0		31
	March 19	98						142.0		9.33	5.24	4.09	0.30	13.8	96.0		28

We found the total base measurements agreed with the summation of the individual cations to within approximately ± 2 per cent and with maximum deviations of -4.5 and $+3.2$ per cent. In figure 2 where the ratios of individual cations to base are plotted, the base represents the average of the total base measurements and the sum of all of the cations. The complete chemical data on the pneumonia patients are given in table 1. Protocols of these patients are in the appendix. In table 2 appear the serum values obtained on ten healthy adults, for the most part members of the medical staff or medical students. Our normal values for total base are considerably lower than those reported by Sunderman, Austin, and Camac in 1926. The 1926 analyses were made on the trichloroacetic acid filtrate from serum obtained from defibrinated blood. The filtrate, after digestion with H_2SO_4 and HNO_3 , was ashed in the final step over a Mecker burner. The analyses of total base reported in this paper were made without removal of the protein, directly on the serum obtained from blood which had been permitted to clot spontaneously before centrifuging. The final step in the ashing was carried out in an electric furnace at dark cherry heat.

In our series of protein fractionations, the conspicuous feature found was the decrease in the albumin concentration not only during the active infection but also in the period of convalescence. The globulin content was increased enough to compensate for the albumin loss in most of the sera excepting before the crisis in cases C14 and C15; in these two patients there was a significant decrease in total protein content of the serum. Values for nonprotein nitrogen were usually high at the time of crisis.

Taking the normal values for base bound by protein as between 16 and 19 m.Eq. per liter, it will be seen in figure 1 that this quantity is usually decreased during the febrile period.

We have shown previously (1) that the total base of serum during the active infection may fall as low as 83 per cent of the mean normal value and that this loss is almost equalled by the decrease in chloride. Sodium normally constitutes between 91 and 94 equivalents per cent of the total base. The sodium level was decreased in all of our cases before and for a considerable period after the crisis and this loss was approximately equal to the decrease in total base.

Our normal values for potassium in serum are slightly lower than those generally given in the literature. They vary from 3.8 to 4.3 mM. per liter with an average of approximately 4.0 mM. per liter. The serum potassium was increased during the febrile period above our normal range in twelve out of eighteen measurements. This increase tended to persist after the crisis. Normally our potassium values represent from 2.4 to 2.9 equivalents per cent of the total base; in pneumonic sera, however, the percentage of potassium rose as high as 4.2 per cent.

In thirteen out of twenty calcium measurements made during the febrile period the calcium level was below the normal range. This invites consideration of the associated changes in serum protein. Csapó and Faubl (25) concluded that 1.89 and 3.89 m.Eq. of Ca were bound respectively to every 100 grams of globulin and albumin. Inasmuch as approximately one half the serum Ca is bound by protein in the non-diffusible form, the tendency to lower calcium concentrations may have been explained in part by the decreased amount of albumin which is uncompensated in Ca binding power by the increased amount of globulin during the course of the disease, however, our measurements do not reveal consistent correlation of serum calcium with the fractions of serum protein.

The magnesium values showed throughout the period of the pneumonic infection, a tendency to scatter about the normal range. Seven analyses were below the lower normal limits, seventeen were within the normal limits, and six were above the upper limit. No particular rise was observed at the time of crisis. Stary and Winternitz (26) showed that there is a non-dialyzable fraction of Mg in serum averaging 28 per cent. Only a very small part of the fluctuation observed in magnesium can be attributed presumably to fluctuations in the serum protein.

Note on gastric secretion during lobar pneumonia

In the course of studies on the chloride metabolism in pneumonia, the absence of free HCl in the gastric contents of two of the patients was noted during the precritical period. We have studied this phenomenon in a series of fourteen gastric analyses on four patients during the course of the disease. Two of the patients (C13 and C14)

received no unusual treatment while the other two (C15 and C16) received a salt mixture containing: KCl, 6 grams; NaHCO₃, 5.9 grams; and MgCl₂, 1.2 gram; and approximately two liters of beverages containing 10 per cent glucose supplementing the diet each day until after the crisis. One half of the salt mixture when added to the ordinary food intake of the patient provides a mean normal salt ration. The dilute alcohol test meal as described by Bloomfield and

TABLE 3
Gastric analyses

Case number	Day of disease	Temperature	Maximal free acidity		Maximal total acidity		Maximal gastric chloride	Serum total base	Serum Cl
			Without histamine	With histamine	Without histamine	With histamine			
		°F.	m.Eq. per liter	m.Eq. per liter	m.Eq. per liter	m.Eq. per liter	m.Eq. per liter	m.Eq. per liter	m.Eq. per liter
C13	-7	104	3		14		104		99
	-4	104	0		7		101	145	101
	0	99	0	0	19	47	112	141	100
	+14	98	30	33	36	42	92	146	99
C14	-4	105	0		11		99	134	97
	-1	102	0	42	11	68			95
	+3	99	9	39	40	67	123	147	96
	+11	98	26	30	57	50	109	144	98
C15	-3	103	19		26		104	136	86
	0	99	37	31	51	51	121	141	91
	+6	98	36	76	55	76	130	142	96
C16	-3	103	0	19	3	35	79	131	92
	0	100	18	34	26	42	94	134	99
	+7	98	49	46	58	53	95		98

Keefer (27) was used for the study of the gastric secretion. Specimens of gastric contents were taken at ten minute intervals for sixty minutes after which time the stomach was emptied and a solution of histamine (0.1 mgm. per 10 kgm. of body weight) was injected subcutaneously. Thereafter, the gastric contents were removed at ten minute intervals for thirty or forty minutes. The specimens were analyzed for free HCl and total acidity by fractional titration using

Topfer's and phenolphthalein indicators and for total chloride by the method used for serum. Total base and chloride of the serum were measured on specimens obtained just before the introduction of the stomach tube.

Convenient values for comparison are the maximal values of free HCl, total acidity, and chloride concentration observed in any one specimen of a given day. These values are given in table 3 together with the average temperature, the day of the disease in relation to the crisis, and the total base and chloride concentration of the serum.

The acidity tended to be lowered until after the crisis. In three of the four subjects there was no significant free HCl without histamine stimulation until after the crises. Histamine induced the appearance of free HCl in two of the three instances in which it was given during the precritical period in a patient who had shown no free HCl after the alcohol meal. Absence of free HCl during the febrile period is to be expected frequently in view of Meyers, Cohen, and Carlson's (28) experimental studies and the clinical observations of others upon gastric contents during fever.

We found no apparent correlation between the changes of either total base or chloride of the serum and the gastric acidity. The highest precritical total gastric acidity was observed in C3 who had at the same time the lowest precritical total base and chloride concentration in the serum. The maximal gastric concentration of chloride showed no variation beyond what may be expected normally in three out of the four subjects.

DISCUSSION

It is difficult to evaluate clearly the significance of changes in the concentration of serum electrolytes until more precise information is obtained concerning their complete metabolism under normal and pathological conditions. This will require a knowledge of the rate of utilization of the electrolytes by the tissues; the rate at which they are excreted by the body; the quantity in each tissue of the body; and a knowledge of the physiological conditions which bring about changes in their distribution. While awaiting such data, however, one may note such occurrences as seem to be correlated with the changes in concentration of electrolytes in the serum and suggest plausible relationships.

One may question first whether the changes observed in the cation concentrations in the serum are secondary to partial inanition and a lowered intake of salts. We have shown in previous studies that negative nitrogen balances are even higher in patients with pneumonia than in fasting subjects and that of the total cations ingested during the active infection in our patients, calcium alone is taken in a quantity similar to that found in an adequate daily ration of salt. From Morgulis' (29) studies of the cation concentration in the serum during the early stages of fasting in dogs, and from Gamble, Ross, and Tisdall's (30) studies of the cation concentrations in the serum of fasting epileptic children, there would appear to be a constancy in the electrolyte concentrations in the serum during inanition in contrast to the changes which we observed in pneumonia. It is possible that the more rapid catabolism of body tissue during the course of pneumonia than during inanition may be related to the change in electrolyte concentration of the serum in pneumonia.

Morgulis observed that when his fasting dogs progressed beyond a twenty per cent loss in weight, there was a decrease in the calcium concentration in the serum. He argues that the calcium bound by protein must be decreased since Csapó and Faubl estimate that approximately twice as much calcium is bound by albumin as by globulin and from the literature there is an apparent increase in the globulin-albumin ratio during starvation. Furthermore, he contends if it be accepted that tetany is due to a decrease in the dialyzable calcium content in the serum, then this quantity must have remained above the tetany limit, since Morgulis' animals showed no evidence of tetany. We believe that the fraction of calcium bound by protein in the serum of our patients may be diminished but, as already noted, our measurements do not reveal consistent correlation of the decrease in the concentration of serum calcium with the decrease in the concentration of serum protein or of the albumin fraction of the serum protein.

The disproportionate changes in the individual cation concentrations constitute evidence against the view that the changes we report can be accounted for by simple dilution of the serum with water. That the phenomena observed, however, may be due partially to the administration of large quantities of water deserves consideration. During the precritical period of pneumonia copious quantities of water

are generally consumed. Greene and Rowntree (31) studied the changes in the blood of dogs after the forced administration of water and found that the serum electrolytes were decreased and that both chloride and sodium concentrations were decreased to a greater extent than could be accounted for by the degree of dilution measured by the increase in plasma volume and by the lowered hemoglobin and serum protein concentrations.

By comparing our studies on the serum with those in the literature on the urinary excretion of bases during pneumonia there is a suggestion that the excretion of each of the electrolyte components may in general be correlated with its concentration in the serum. Thus during the precritical period of pneumonia there appears to be a decreased concentration in the serum of sodium and calcium and there is reported a decreased excretion of these ions in the urine; there is an increased concentration of potassium in the serum and there is reported an increase of potassium in the urinary excretion; the magnesium concentration in the serum appears to fluctuate in either direction from the normal, and the same is reported of its excretion (4, 7).

I am indebted to Miss Priscilla Williams for assistance in the analytical work.

CONCLUSIONS

The total base and protein components of the serum have been studied before and after the crisis in patients suffering with lobar pneumonia.

The sodium concentration in the serum was decreased in all of the cases studied before and for a considerable period after the crisis. The decreased concentration of sodium was approximately equal to the decreased concentration of total base.

The potassium concentration in the serum was increased in over one half of the determinations made before the crisis. This increase tended to persist after the crisis.

The calcium values were generally decreased during the febrile period.

Magnesium values were scattered about the normal range throughout the period of infection.

The albumin concentration in the serum was diminished not only during the period of active infection but also during convalescence. The globulin fraction was usually increased, so that the total protein tended to remain within the normal or lower normal limits.

Base bound by protein was usually decreased during the febrile period.

From studies of fourteen gastric analyses on four patients suffering with lobar pneumonia it was noted that:

1. The gastric acidity tended to be lowered until after the crisis.
2. When lowered it was generally increased by histamine stimulation.
3. There was no apparent correlation of either total base or chloride of the serum with the gastric acidity.

PROTOCOLS

Case C1 (Number 14743). Colored, male, age 20, janitor. The patient was admitted with consolidation of the right lower lobe. He was dyspneic, cyanotic, and jaundiced. Sputum yielded a Type I pneumococcus. The highest leukocyte count occurred just before the crisis, and was 40,000. Blood Wassermann was positive. The patient gave a history of having had lobar pneumonia confined to the right lower lobe two years previously which was followed by an empyema. Convalescence was uneventful.

Case C2 (Number 14860). Colored, male, age 23, laborer. The patient was admitted to the hospital with a lobar pneumonia confined to the left lower lobe. Respirations were quite labored and restricted over the left lower chest. A pericardial friction was audible over the precordium. The patient's temperature fell by crisis on the eighth day of the disease. However, there were daily rises in temperature to 100° until after the eighteenth day of the disease. Leukocyte count varied from 9000 to 20,000. Sputum yielded a Type I pneumococcus. Blood Wassermann was positive.

Case C3 (Number 15328). Irish, male, age 57, sailor. The patient entered the hospital with a history of exposure on shipboard followed by severe pain in the right chest of 5 days' duration. Physical examination revealed a consolidation of the right lower lobe. Temperature fell by crisis on the eighth day of the disease. Highest leukocyte count was 24,000 with 80 per cent polymorphonuclears. Convalescence was uneventful.

Case C4 (Number 15085). Colored, male, age 30, cook. The patient had an upper right lobar pneumonia with a crisis on the fifth day of the disease. Group

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IV pneumococci were isolated in the sputum. Leukocyte count on admission was 23,000 with 86 per cent polymorphonuclears. Convalescence was uneventful.

Case C5 (Number 15210). Hebrew, male, age 18, clerk. The patient was admitted on the sixth day of the disease with signs of consolidation of the right lower lobe. Temperature became normal by lysis four days after admission. X-ray evidence was suggestive of active pulmonary tuberculosis but the clinical course proved it to be otherwise. Sputum examination did not reveal the presence of any tubercle bacilli. Leukocyte counts varied from 17,500 to 10,000.

Case C6 (Number 14965). Colored, male, age 24, laborer. The onset of the disease began with a peritonsillar abscess. Physical examination pointed to consolidation of the right lower lobe. Lung abscess was considered but later excluded. Postural drainage proved unsuccessful. Sputum yielded Group IV pneumococci and Friedlander's bacilli. Convalescence was uneventful.

Case C7 (Number 15447). Colored, male, age 34, laborer. The patient entered the hospital with a typical lobar pneumonia confined to the right lower lobe. Crisis occurred on the fifth day of the disease. Leukocyte count on admission was 16,700. The sputum yielded Group IV pneumococci. Blood Wassermann was positive. Convalescence was uneventful.

Case C8 (Number 15499). Colored, male, age 30. The patient was first studied on the seventh day of a left lower lobar pneumonia. Pleural frictions were audible in the left axilla. The patient did not become afebrile until after the twenty-fourth day of the disease. Sputum yielded a Type III pneumococcus. Leukocyte count on admission was 30,000 with 80 per cent polymorphonuclears. Convalescence was protracted but uncomplicated.

Case C9 (Number 15564). Italian, male, age 34, laborer. The patient entered the hospital with a major complaint of right abdominal pain. Physical examination revealed a right lower lobar pneumonia and evidences of a frontal sinus infection. Leukocytes ranged from 29,000 to 12,000. Wassermann was positive. Sputum contained Group IV pneumococci. Patient did not become afebrile until 12 days after admission.

Case C10 (Number 15645). White, male, age 63, machinist. On admission patient was apneic, jaundiced, and cyanotic. Physical signs gave evidence of consolidation of the right lower lobe. Sputum yielded Group IV pneumococci and Friedlander's bacilli. The patient died two days after admission.

Case C11, (Number 15663). Hebrew, female, age 15, student. The patient on admission was prostrated, cyanotic, and had consolidation of the right lung. She developed pulmonary edema in twenty-four hours after admission and died. Leukocyte count on admission was 5,000.

Case C12, (Number 15862). Colored, male, age 28, laborer. Patient was admitted to the hospital in a critical condition, apneic, perspiring profusely, and comatose. Percussion note over right chest was impaired, over which area rhonchi were audible. Leukocytes ranged from 18,000 to 19,000. Sputum contained Group IV pneumococci. Wassermann was strongly positive. The patient died on what was regarded as the ninth day of his disease.

Case C13, (Number 9352). Colored, male, age 23, laborer. The patient entered on the fourth day of the disease and showed evidence of consolidation of the right lower lobe. The crisis occurred on the twelfth day of the disease. Highest leukocyte count occurred at the time of crisis and was 27,000. Sputum yielded Group IV pneumococci. Convalescence was uneventful.

Case C14 (Number 9393). White, male, age 30, laborer. The study was begun on the sixth day of the disease. The patient had a right upper and lower lobar pneumonia. The urine contained a cloud of albumin and granular casts during the febrile period. Crisis occurred on the ninth day of the disease. A Type II pneumococcus was recovered from sputum. Convalescence was protracted for four weeks.

Case C15, (Number 9750). White, male, age 31, chauffeur. The patient was brought into the hospital in a delirious state. He had been sick at home complaining of severe pain in left chest, coughing, hemoptysis and high fever for six days previous to admission. Physical examination revealed consolidation of left lower lobe and congestion of right lower lobe. On the seventh day after admission, temperature and pulse fell by crisis and the patient made an uneventful recovery. Sputum yielded both Group IV pneumococci and influenza bacilli. Leukocytes ranged from 9,600 to 18,000.

Case C16, (Number 10025). Colored, male, age 25, laborer. The patient entered the hospital with evidences of incomplete consolidation in both lower lobes and complete consolidation in the right upper lobe. Crisis occurred on the seventh day of the disease. No pneumococci were isolated from the sputum. Convalescence was uneventful. Wassermann and Kahn reactions were strongly positive.

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THE ELIMINATION OF ETHYL IODIDE AFTER INHALATION AND ITS RELATION TO THERAPEUTIC ADMINISTRATION¹

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Previous communications have demonstrated the advantages of inhalation of ethyl iodide in treating certain diseases (1, 2). Two hundred patients with epidermophytosis, tinea capitis, tinea favosa, monilia infections, cryptococcosis epidermica, blastomycosis and other mycotic infections have been treated by means of inhalations of ethyl iodide. Many of these patients, treated for a period of years with unsatisfactory results by the methods hitherto available, have shown striking improvement and even complete recovery. No reports of the therapeutic use of ethyl iodide by other investigators were available in the literature. The purpose of the present investigation was to study the elimination of ethyl iodide after inhalation in order to establish the optimum dosage and frequency of treatment, and to gain knowledge concerning the mechanism of its action.

AMOUNT OF ETHYL IODIDE RETAINED IN BODY AT THE END OF INHALATION PERIOD

The first object of the study was to measure the amount of ethyl iodide remaining in the body at the termination of the inhalation of the ethyl iodide vapor.

The ethyl iodide² was weighed, or was measured by means of a

¹ This investigation was aided by a grant from the DeLamar Mobile Research Fund of Harvard University.

² All ethyl iodide used in this investigation was obtained by repurification of Merck's ethyl iodide according to directions given by Fisher (11). Ethyl iodide which has been properly prepared is colorless, has a specific gravity of 1.94 at 15°C., a boiling point of 72.2°C. and is phosphorus-free. It should be stored in a brown bottle. The addition of a globule of mercury will keep the product colorless.

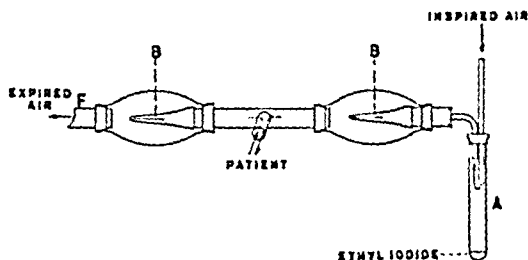


FIG. 1. DIAGRAM OF APPARATUS USED IN THE INVESTIGATION

The inspired air is drawn through test tube *A* containing the ethyl iodide. The concentration of ethyl iodide in the inspired air can be varied by altering the degree to which the straight glass tube projects into the test tube. Rebreathing is prevented by the use of flutter valves *B, B*. The expired air passes through outlet tube *F*. For clinical purposes a simpler, more practical apparatus has been devised.

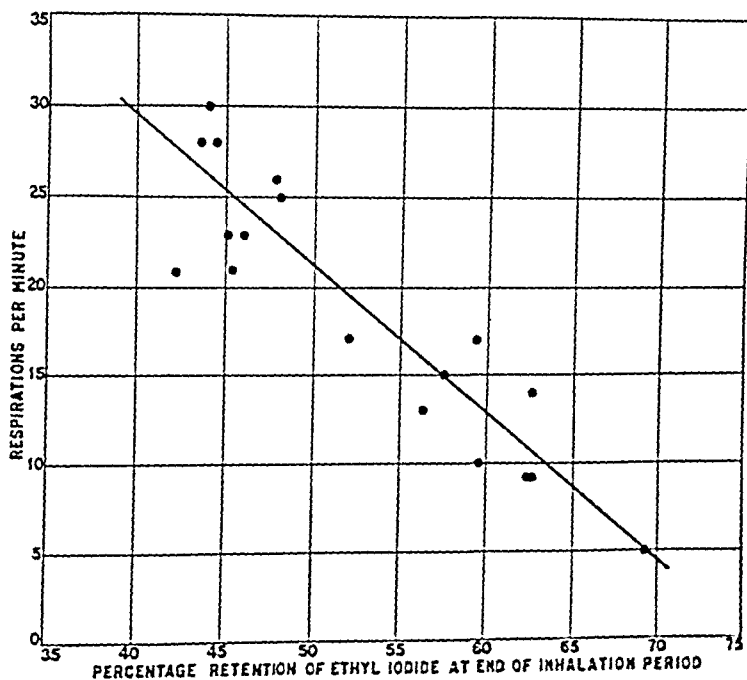


FIG. 2. THE RELATION BETWEEN THE RESPIRATORY RATE AND THE PERCENTAGE OF ETHYL IODIDE RETAINED WITHIN THE BODY AT THE IMMEDIATE CONCLUSION OF THE INHALATION PERIOD

United States Bureau of Standards pipette. The desired amount was transferred to a test tube (*A*, fig. 1). The apparatus was constructed to permit partial saturation of the inspiratory current of air with ethyl iodide vapor. The two way flutter valve system *B* (fig. 1)

TABLE 1

Amount of ethyl iodide retained in body at end of inhalation period

Measure- ment number	Subject	Date	Dura- tion of inhalation	Respira- tory minute volume	Respira- tions per minute	Amount of ethyl iodide inhaled	Amount exhaled during period of inhalation	Amount retained at end of treatment	Per cent of total retained at end of treat- ment
		1930	minutes	liters		grams	grams	grams	per cent
1	S. B.	January 23	10	10.2	21	1.94	1.12	0.82	42.2
2	S. B.	January 27	26	9.0	23	1.93	1.06	0.87	45.1
3	S. B.	January 30	39	8.5	23	3.64	1.96	1.68	46.1
4	S. B.	February 4	38	8.7	21	5.76	3.14	2.62	45.5
5	M. K.	February 4	35	8.8	30	3.82	2.14	1.68	44.0
6	H. C.	February 6	20	10.6	28	1.94	1.08	0.86	44.3
7	H. C.	February 10	30	11.3	28	3.68	2.08	1.60	43.5
8	H. C.	February 13	33	9.8	25	5.00	2.60	2.40	48.0
9	E. R.	February 3	32	9.0	26	4.96	2.58	2.38	48.0
10	R. A.	January 31	25	11.9	17	4.10	2.00	2.10	51.3
11	J. E.	January 31	40	7.4	13	5.44	2.38	3.06	56.3
12	M. G.	January 24	25	7.2	10	3.88	1.57	2.31	59.6
13	M. G.	January 27	43	7.8	15	3.30	1.40	1.90	57.6
14	H. D.	January 29	26	10.8	17	5.82	2.38	3.44	59.2
15	M. V.	February 14	17	8.0	14	1.94	0.72	1.22	62.9
16	M. V.	February 18	22	6.2	9	1.94	0.73	1.21	62.4
17	M. V.	February 24	47	6.3	9	5.82	2.17	3.65	62.8
18	C. V.	March 14	60	5.5	5	5.82	1.81	4.01	69.0

communicated directly with a Tissot spirometer of four hundred liter capacity in which the total amount of expired air was collected and measured. The inside of the spirometer was coated with red lead. Gas samples of 250 cc. were taken from the spirometer and the

ethyl iodide content measured according to the method of Starr and Gamble (3). We have been able by this method to recover known amounts of ethyl iodide with a high degree of accuracy. The total amount of exhaled ethyl iodide could then be calculated. The difference between the amount of ethyl iodide volatilized in the inspired air and the amount exhaled represented the amount remaining within the body at the immediate conclusion of a treatment.

The results of eighteen measurements in ten different subjects are shown in table 1. The amount of ethyl iodide inhaled varied from 1.93 to 5.82 grams. In different individuals, the percentage of ethyl iodide retained in the body varied from 42.2 to 69.0 per cent of that inhaled; but, in a given individual, the percentage retained in successive experiments was remarkably constant, regardless of the amount inhaled. In subject S. B., for example, 1.93 to 5.76 grams of ethyl iodide were inhaled but the percentage retained varied only from 42.2 to 46.1 per cent. The percentage of ethyl iodide retained was not affected by the length of time taken for inhalation (table 1). The percentage retained depended to a large extent, however, on the respiratory rate; the lower the respiratory rate, the greater the percentage retention (fig. 2). With lower respiratory rates more time is probably available for effective absorption of ethyl iodide by the blood of the alveolar capillaries. In subjects with normal respiratory rates, the average retention of ethyl iodide in the body at the conclusion of treatment was 55 per cent of the total amount inhaled.

THE AMOUNT OF ETHYL IODIDE EXHALED AFTER END OF INHALATION PERIOD

Besides the ethyl iodide exhaled during treatment, additional amounts were exhaled after treatment. To study this latter factor the following method was used. Ethyl iodide was administered according to the procedure described above except that the outlet tube *F* (fig. 1) was led to the air outside the laboratory to prevent reinhalation of the ethyl iodide exhaled during the treatment. As soon as all the ethyl iodide in test tube (*A*) (fig. 1) had been vaporized, the test tube was removed and the expired air collected in a Tissot spirometer at appropriate intervals for five or ten minute periods. The total volume of expired air during each period was measured,

and samples of 250 to 1000 cc. were taken and analyzed. From these data, the total amount of ethyl iodide exhaled during any given period of time as well as the average number of milligrams of ethyl iodide exhaled per minute could be calculated. By means of these results curves of excretion were plotted (fig. 3). The final portions of the curves (dotted line) of excretion were estimated since the concentration of ethyl iodide became too small to be measured.

Indirect evidence in regard to the approximate accuracy of the terminal portions of the curves was obtained by analysis of samples of alveolar air. The concentration of ethyl iodide in the alveolar

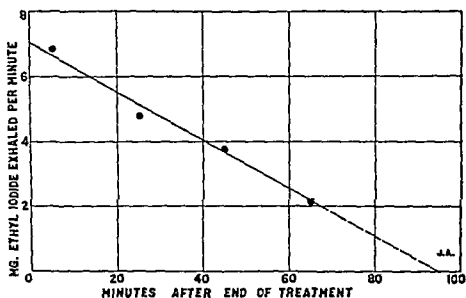


FIG. 3. THE RATE AT WHICH ETHYL IODIDE IS GIVEN OFF IN THE EXPIRED AIR FOLLOWING TREATMENT

air would be higher than that of the total expired air inasmuch as the expired air collected in the usual manner represents alveolar air diluted by air of the dead space. The absence of ethyl iodide in the alveolar air collected after the projected portion of the curve had reached zero would be confirmatory evidence of the accuracy of this part of the graph. Ninety to 120 minutes after the conclusion of treatment, samples were obtained by collecting the air of successive forced expirations until a total of one liter was obtained. Four analyses in two subjects showed no detectable amounts of ethyl iodide.

The total amount of ethyl iodide exhaled could, therefore, be calculated by integrating the area under the curve. Thirteen series of measurements were made in eight subjects (table 2). The length of

ELIMINATION OF ETHYL IODIDE

time ethyl iodide continued to be exhaled following treatment and the total amount of ethyl iodide exhaled were apparently unrelated to such factors as dosage, duration of treatment or respiratory rate of the patient. The average amount of ethyl iodide exhaled after the end of the inhalation period was 0.35 gram or 9 per cent of the mean total dose.

TABLE 2
The amount of ethyl iodide exhaled after end of inhalation period and related data

Measure- ment number	Name	Amount ethyl iodide inhaled	Duration of inhalation period	Respirations per minute	Time after inhalation when ethyl iodide ceases to be exhaled	Total amount of ethyl iodide exhaled after end of inhalation period
		<i>grams</i>	<i>minutes</i>		<i>minutes</i>	<i>grams</i>
1	M. V.	1.94	17	16	107	0.44
2	C. V.	1.94	23	5	98	0.22
3	H. N.	1.94	24	16	82	0.20
4	W. M.	1.94	20	25	60	0.36
5	M. V.	3.88	35	11	45	0.42
6	M. V.	3.88	30	9	62	0.36
7	R. P.	3.88	38	22	97	0.18
8	S. B.	5.67	39	29	117	0.54
9	J. E.	5.82	36	13	68	0.45
10	M. V.	5.82	47	16	100	0.55
11	J. A.	5.82	45	22	94	0.33
12	C. V.	5.82	60	5	103	0.25
13	C. V.	5.82	50	6	49	0.29

EXCRETION OF IODIDE IN THE URINE

The next object of the investigation was to learn in what manner and at what rate the remainder of the ethyl iodide was excreted. Ten specimens of urine collected immediately following treatment were analyzed for ethyl iodide by the method of Starr and Gamble (4). No free ethyl iodide could be detected.

Other investigators (5, 6, 7, etc.) have shown that the major portion of inorganic iodine salts administered orally, rectally, or intravenously is excreted as iodide in the urine. In the present investigation all urine was collected over a period of days following treatment, the time of voiding, the volume and the iodide content

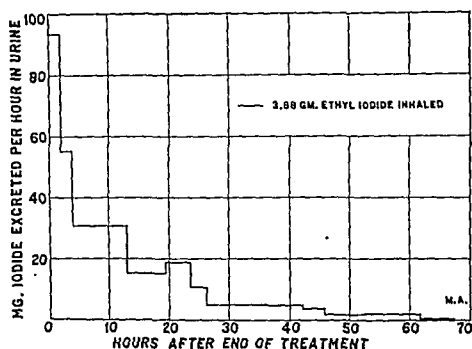


FIG. 4. EXCRETION PER HOUR OF IODIDE IN THE URINE AFTER SUBJECT (M. A.) HAD INHALED 3.88 GRAMS ETHYL IODIDE

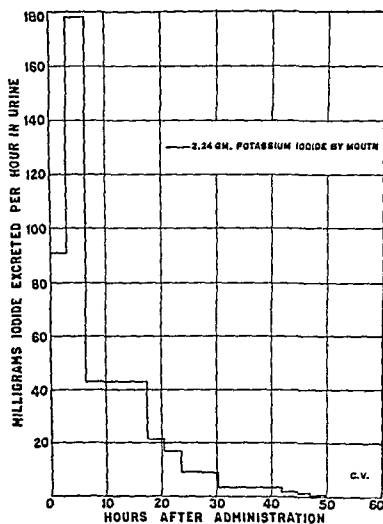


FIG. 5. EXCRETION PER HOUR OF IODIDE IN THE URINE AFTER SUBJECT (C. V.) RECEIVED 2.24 GRAMS OF POTASSIUM IODIDE BY MOUTH

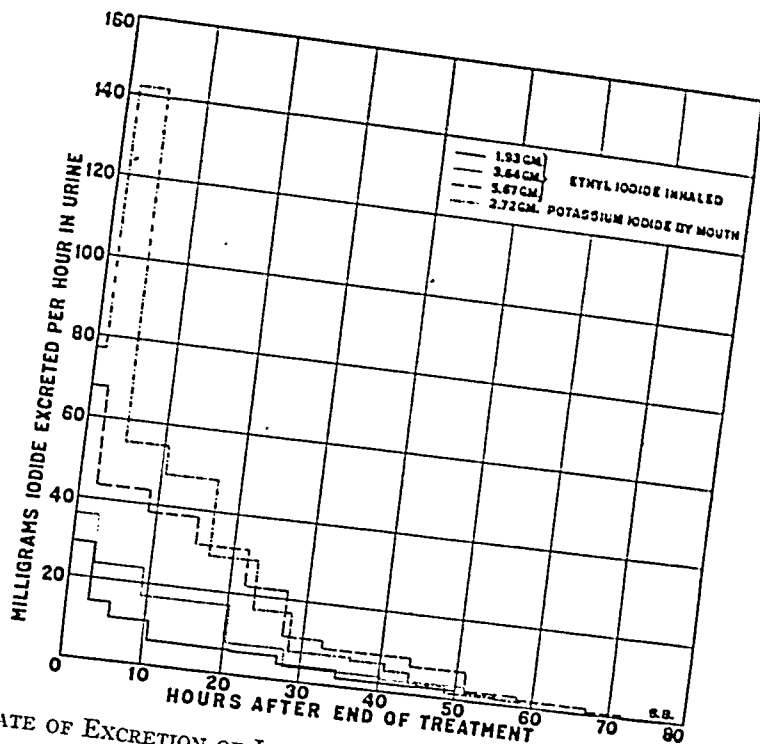


FIG. 6. RATE OF EXCRETION OF IODIDE IN THE URINE IN SUBJECT (S. B.) AFTER INHALATION OF VARIOUS AMOUNTS OF ETHYL IODIDE AND AFTER POTASSIUM IODIDE BY MOUTH

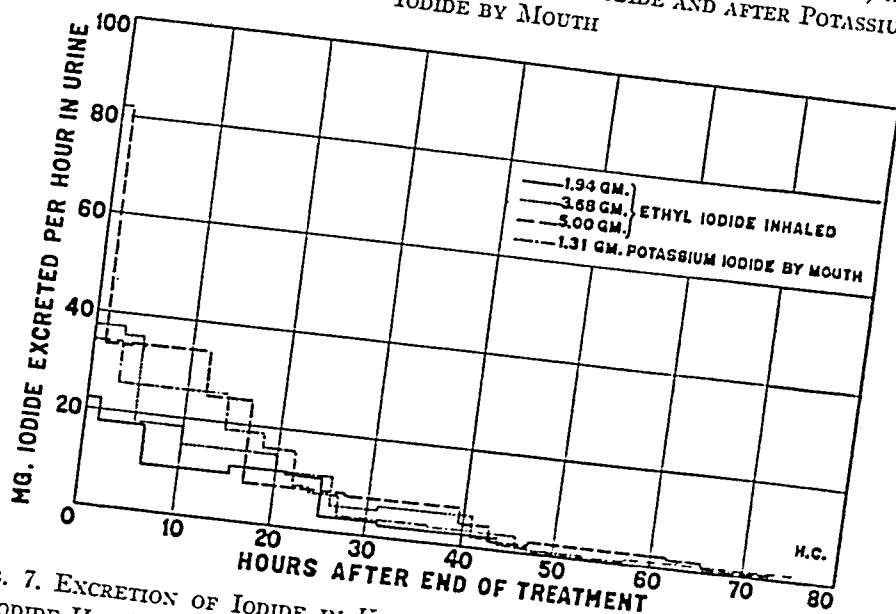


FIG. 7. EXCRETION OF IODIDE IN URINE AFTER VARIOUS AMOUNTS OF ETHYL IODIDE HAD BEEN INHALED AND AFTER POTASSIUM IODIDE BY MOUTH HAD BEEN GIVEN TO SUBJECT (H. C.)

of each specimen being recorded. From these data the hourly excretion of iodide was calculated. The iodide excreted in the urine after ethyl iodide inhalation was compared with that following the administration of similar doses of potassium iodide by mouth. The method used for measuring the iodide in the urine was substan-

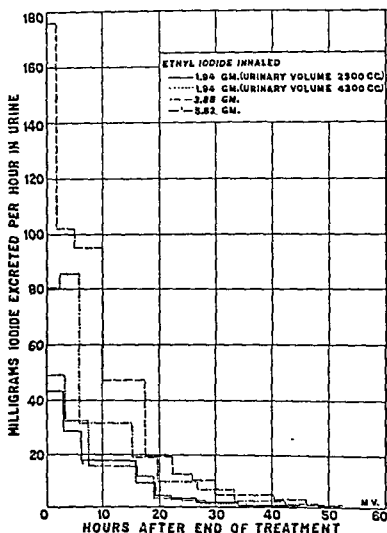


FIG. 8. EXCRETION OF IODIDE IN URINE AFTER VARIOUS DOSES OF ETHYL IODIDE HAD BEEN GIVEN TO SUBJECT (M. V.)

The effect of urinary volume on excretion was studied by doubling the fluid intake after the second dose of 1.94 grams of ethyl iodide had been taken. The two curves of excretion closely correspond.

tially that of Bolliger (8). Charcoal was substituted for Lloyd's reagent. The oxidizing reagent consisted of a ten per cent solution of sodium nitrite in concentrated sulphuric acid. Standards were made containing 0.5 to 6.0 mgm. of iodine in 25 cc. of CCl_4 . Small test tubes of uniform diameter were employed in the colorimetric comparison of the standard and unknown solutions.

Figures 4, 5, 6, 7 and 8 show the hourly excretion of iodide in the urines of five subjects after the administration of various amounts of ethyl iodide by inhalation and of potassium iodide by mouth. The curves of iodide excretion after ethyl iodide and potassium iodide are of the same general type, the rate and duration of excretion being strikingly similar.

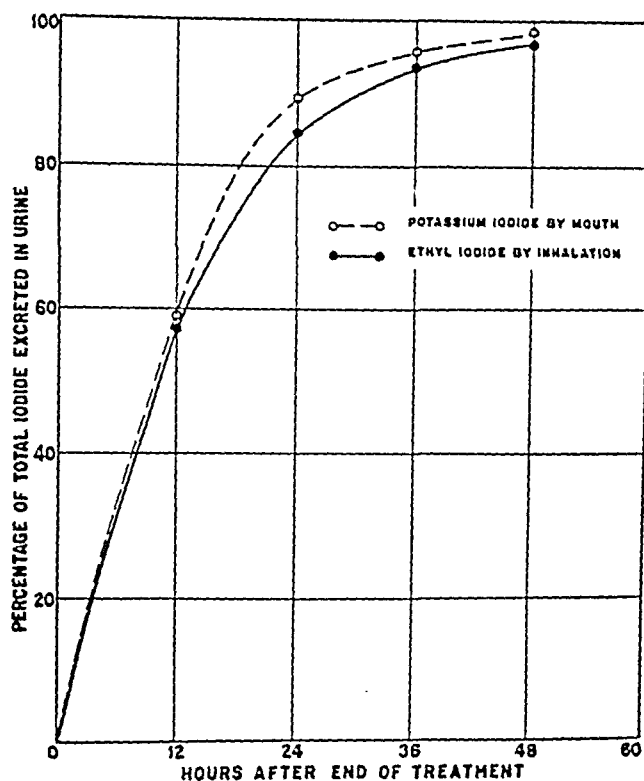


FIG. 9. THE AVERAGE RATE OF IODIDE EXCRETION EXPRESSED AS PERCENTAGE OF THE TOTAL EXCRETED IN THE URINE

Curves showing the percentage excretion during successive 12-hour periods following treatment varied but slightly in all subjects regardless of the dosage. The average rate of iodide excretion expressed as the percentage of the total excreted in the urine is shown in figure 9.

In a given patient, urinary excretion of iodide ceased after approximately the same time interval regardless of the amount of ethyl iodide inhaled (table 3). The duration of excretion was not altered

appreciably by doubling the fluid intake (solid and dotted lines, figure 8). That the volume of urine did not affect the rate of excretion

TABLE 3
Measurements of excretion of iodide in urine

Measurement number	Subject	Date	A Amount of ethyl iodide inhaled	B Amount of ethyl iodide retained during inhalation	C Amount of ethyl iodide not excreted by lungs (calculated)	D Iodide equivalent of ethyl iodide not excreted by lungs	E Iodide excreted by kidneys	F Percentage of retained iodide excreted in urine $E \div D \times 100$	G Time after inhalation when rate of urinary iodide excretion becomes less than 0.5 mgm. per hour	H Time after inhalation when no trace of iodide can be detected in urine
		1930	grams	grams	grams	grams	grams	per cent	hours	hours
1	S. B.	January 27	1.93	0.87	0.52	0.42	0.32	76	>50	
2	S. B.	January 30	3.64	1.68	1.33	1.08	0.58	54	65	113
3	S. B.	February 10	5.82	2.64	2.29	1.86	1.35	73	67	114
4	S. B.	February 19	5.67	2.58	2.23	1.81	1.23	68	72	124
5	H. C.	February 6	1.94	0.86	0.51	0.41	0.35	85	>44	71
6	H. C.	February 10	3.68	1.60	1.25	1.02	0.59	58	53	>77
7	H. C.	February 13	5.00	2.44	2.09	1.70	0.78		73	109
8	M. V.	February 14	1.94	1.22	0.87	0.71	0.45	64	45	94
9	M. V.	February 18	1.94	1.21	0.86	0.70	0.51	73	40	71
10	M. V.	April 3	3.88	2.43	2.08	1.69	1.02	61	51	80
11	M. V.	February 24	5.82	3.65	3.30	2.68	1.71	64	52	95

TABLE 4
Percentage excretion of iodide in urine after administration of potassium iodide

Measurement number	Subject	Amount potassium iodide administered by mouth	Iodide equivalent	Amount iodide excreted in urine	Urinary iodide excretion calculated as percentage of intake
		grams	grams	grams	per cent
1	H. C.	1.31	1.00	0.64	64
2	C. V.	2.24	1.71	1.42	83
3	S. B.	2.72	2.08	1.64	79

was also shown by the fact that S. B. continued to excrete iodide in the urine for a somewhat greater length of time than H. C. or M. V.

(table 3) although the urinary volume of the latter subjects was approximately one-half as great.

The total amounts of iodide excreted in the urine of three patients after doses of potassium iodide by mouth are given in table 4. Of the total iodide ingested an average of 75 per cent was excreted by the kidneys. These results are in accord with observations of others (5, 6, 7, etc.). The remainder of the iodide, according to these investigators, is excreted by the intestines, the skin, and in the sputum. Of the total amount of ethyl iodide not eliminated by the lungs, an average of 68 per cent (table 3) is excreted by the kidneys as iodide. It is reasonable to conclude that the remainder of the iodide is excreted in the same manner as mentioned above. Excluding the ethyl

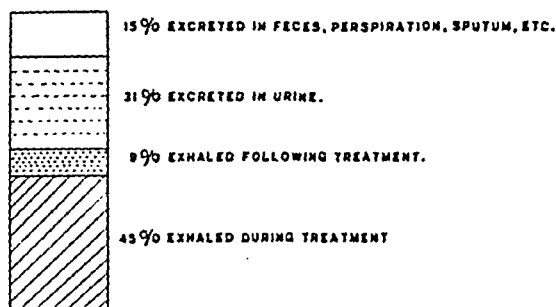


FIG. 10. DIAGRAMMATIC REPRESENTATION OF THE FATE OF ETHYL IODIDE ORIGINALLY INHALED

iodide given off by the lungs, our results indicate the similarity in the excretion of iodides, whether administered originally as the organic compound, ethyl iodide; or as an inorganic salt, potassium iodide.

In brief, one may conclude that after 1.0 to 3.0 cc. of ethyl iodide are inhaled, an average of 45 per cent is immediately exhaled during treatment, 9 per cent is exhaled during the first 2 hours after treatment and 31 per cent is excreted by the kidneys. This accounts for 85 per cent of the total dose given, the other 15 per cent being excreted in all probability by the intestines, skin, and in the sputum (fig. 10).

DISCUSSION

Frequency and amount of dosage. The action of a drug depends in large part on its concentration within the body. It is consequently

desirable to maintain a high level of concentration of the drug in the body by administering sufficient amounts at appropriate intervals to offset excretion of the substance. Elimination of ethyl iodide takes place through the lungs, the kidneys, the gastro-intestinal tract, and in the sputum. The results of the present investigation demonstrate that a total of 54 per cent of the inhaled ethyl iodide is exhaled during treatment and within the first few hours immediately following treatment. Measurements of the elimination of iodide in the urine after inhalation of ethyl iodide offer important evidence regarding the amount remaining within the body since 68 per cent of the ethyl iodide not exhaled during and immediately following treatment, is excreted as iodide in the urine (table 3). The rate of excretion is greatest immediately following treatment and decreases rapidly. Approximately 85 per cent of the total urinary iodide is excreted within the first 24 hours and approximately 97 per cent is excreted by the end of 48 hours. This indicates that daily repetition of the dosage is desirable to maintain a high level of iodide within the body; that is to say, a given dose repeated daily will maintain a higher level of concentration than twice that dose given every other day. It should be noted, however, that since 15 per cent or more remains within the body, daily repetition will lead to accumulation and, ultimately to toxic effects. To prevent such an occurrence, the daily dosage could be reduced on each successive day, but practically, the same purpose may be achieved by omitting treatment every third or fourth day. Forty-eight hours will then elapse between two treatments, an interval of time sufficiently great to permit almost complete elimination of the accumulated iodide. The amount of the daily dosage of ethyl iodide must depend largely on the therapeutic results and the absence of toxic effects as observed clinically. Clinical experience up to the present indicates that daily doses of 4 grams are efficacious but further experience is necessary to establish this more precisely. Future communications will give further information regarding this point.

Influence of fluid and food intake on excretion of iodide. The water intake during treatment need not be regulated since the excretion of iodide seems to be independent of the fluid ingested (fig. 8). The influence of diet was not studied since the iodide content of food is so

small that the effect may be considered unimportant for these purposes. It is probable, that in the presence of toxicity, increased amounts of sodium chloride in the diet will facilitate excretion.

Contraindications to the use of ethyl iodide. Impaired excretory function of the kidney will probably favor retention of iodide and the appearance of toxic effects. We have therefore been particularly careful to avoid treating subjects with evidence of nephritis. The urine of all subjects was examined previous to and following treatment in order to exclude any possible untoward effects on kidney function. No evidence of renal irritation was observed. Ethyl iodide was not administered to any patients with symptoms or signs of thyrotoxicosis.

Mechanism of action of ethyl iodide. The use of ethyl iodide was originally suggested because it is an unstable compound which can be absorbed directly into the arterial circulation and so reach the peripheral tissues in high concentration. Ethyl iodide is only slightly soluble in water so that intravenous administration would be inadvisable. If given intravenously a part of the ethyl iodide would, moreover, be given off immediately by the lungs before it reached the arterial blood. The investigations of Henderson and Haggard (9), and of Starr and Gamble (10) indicate that more than three-quarters of inhaled ethyl iodide is immediately hydrolyzed in the arterial and capillary blood before it reaches the veins. Some of the ethyl iodide, however, passes into the tissues during inhalation and accumulates there in increasing amounts, as shown by the fact that, after ethyl iodide inhalation is discontinued, venous concentration of ethyl iodide exceeds arterial concentration. The exact changes that occur in the peripheral blood are still obscure. Minute amounts of free iodine may conceivably be liberated. We have taken blood from patients before and after treatment to learn whether the growth of fungi was less in the latter blood but no significant difference was noted. It is of interest to note that a toxic rash which appeared in a few patients receiving repeated large doses of ethyl iodide was first observed in the diseased areas rather than according to the conventional distribution. The fact that ethyl iodide is a lipid solvent may also explain in part its striking therapeutic efficacy.

Investigations are planned to study the value of ethyl iodide in other

diseases and the advantages to be obtained from quantitative inhalation therapy.

SUMMARY AND CONCLUSIONS

1. The fate of ethyl iodide in the body has been studied after the inhalation of amounts found strikingly effective in treating more than 200 patients with certain skin diseases.

2. The results of this investigation offer a rational basis for determining the optimum dosage and the frequency of treatment.

3. After 1.0 to 3.0 cc. of ethyl iodide are introduced into the inspired air, an average of 45 per cent is immediately exhaled during the period of treatment, an average of 9 per cent is exhaled during the first two hours after treatment and 31 per cent is excreted by the kidneys. This accounts for 85 per cent of the total dose given, the other 15 per cent being excreted in all probability by the intestines, skin, and in the sputum.

4. Of the ethyl iodide excreted as iodide in the urine, approximately 85 per cent is excreted within the first 24 hours and approximately 97 per cent is excreted by the end of 48 hours.

5. The results indicate that daily repetition of the treatment is desirable to maintain a high level of iodide within the body.

6. To avoid accumulation of the drug and possible toxic effects, treatment should be omitted every third or fourth day.

7. Within the range of therapeutic dosage, the percentage of ethyl iodide retained in the body is independent of the size of the dose, of the length of time taken for inhalation and of the fluid intake and output.

8. The mechanism of action of ethyl iodide and the contraindications to its use are discussed.

We are indebted to Miss Mary Vastine for technical assistance.

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NORMAL STANDARDS OF GASTRIC FUNCTION

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The establishment of normal standards of gastric function is a prerequisite to the recognition of abnormal conditions. Such standards have heretofore been inadequate, with the result that studies of stomach secretion have fallen into disfavor in the clinic. As we pointed out in a previous paper (1), a test of function must be based on certain general criteria: (1) the test must be applied under standard conditions, (2) it must impose a load on the function under consideration, (3) it must be capable of identical repetition and (4) it must actually yield information which experience shows to be useful. Reasons why the gastric test-meals in ordinary use are unsatisfactory were also exposed (1), and it was pointed out that measurement of the volume and acidity of the pure gastric juice, after stimulation by histamine, constituted the most fruitful clinical procedure at present available for studying the secretory function of the stomach. The technique of the method was described, and it was shown that if the total gastric juice is collected over successive ten-minute periods after stimulation, the greatest quantity is usually produced in the twenty to thirty minute period, and the acidity usually reaches a maximum at this time (2). These two values, therefore, the greatest ten-minute secretory volume and the highest acidity attained, make up the essential elements in an assay of the secretory capability of a given stomach under definite conditions.

Determination of these factors in an adequate series of normal people would furnish data with which deviations in disease might properly be compared and from which useful conclusions could be drawn. Some years ago an attempt was made to set up standards along these lines on the basis of data obtained by alcohol test meals (3, 4). While useful information was obtained there were, as a result of the inadequacy of alcohol as a stimulus to gastric secretion, some errors which

have since been recognized; in brief, a good many "normal" people who failed to secrete acid have since been shown to be capable of doing so by the application of histamine. Hence the erroneous conclusion was drawn that gastric acidity is much lower in the old than in the young, a finding which the present observations have modified. The conclusion that, on the whole, volume of secretion lessens considerably with age is however sustained, as will be pointed out below.

MATERIAL

The selection of the normal people, who were to serve as subjects for the derivation of standards, immediately presented a serious problem. It seemed proper that the selection should be made before the test was carried out, and we promptly encountered the difficulty of finding people who had always been entirely free of digestive symptoms. However, such a series was assembled and the tests were carried out; but despite the absence of any historical or physical evidence of gastric disorder, it became evident, on the basis of the secretory studies, that some of the subjects did not possess normal stomachs. This was especially so of certain individuals who failed to secrete acid, or secreted it only in minimal amounts, after histamine stimulation. The exact significance of such findings is not clear, but in view of the presumption that an organic lesion of the mucosa must exist in these cases they were arbitrarily discarded from the series. This subject will be discussed in another paper. In brief, then, people who had no digestive symptoms, and who secreted a juice with free hydrochloric acid of over 10 were considered normal. It also seemed undesirable to base the standards on findings in any highly selected group such as medical students, with whom one could not fairly compare the miscellaneous run of people of all ages seen in medical practice. We used, therefore, hospital patients provided the above specifications were fulfilled and provided there was no acute, febrile or cachectic condition or any disorder which presumably might disturb gastric function such as cardiac decompensation.

METHODS

The test procedure has already been outlined (1, 2). Briefly, a small tube was introduced into the stomach under fasting basal con-

ditions. After the fasting juice had been withdrawn 0.1 mgm. of histamine (ergamine) per 10 kgm. of body weight was injected hypodermically. The total secretions were then aspirated over successive ten-minute periods until the flow of juice subsided to the basal level (usually one hour). The greatest ten-minute volume of secretion

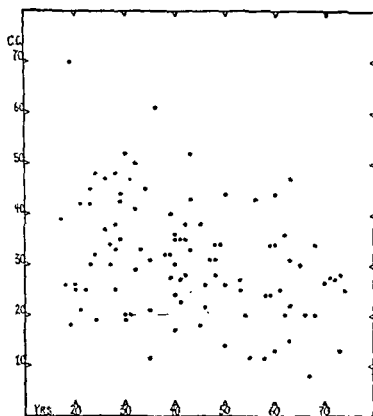


CHART 1. HIGHEST TEN-MINUTE SECRETORY VOLUME IN EACH CASE (CC.)
PLOTTED AGAINST AGE OF SUBJECT

and the highest titratable acidity attained were considered indices of stomach function, and they furnish the data which are herewith reported.

RESULTS

Volume of secretion. In chart 1 each dot represents the greatest ten-minute volume of gastric secretion, after histamine stimulation, in a different person. The wide variations are striking, and make it evident that no very narrow standards of normality can be set up. However, as shown by the distribution curve¹ (chart 2), about one-

¹ No attempt has been made to subject the material to accurate mathematical analysis. It is evident, however, that the distribution of secretory volumes approaches a normal curve whereas the distribution curve of acidity is skewed (chart 5).

half of the determinations fell within the limits of 21 to 35 cc. We have made no attempt to correlate the findings with any special habitus or type of person, except in so far as age has been considered, and a further inspection of chart 1 shows that most of the larger volumes are found in young people whereas the smaller volumes are mainly from those over fifty years of age. If one averages the secretory volumes for the various decades, the fact that less gastric juice is

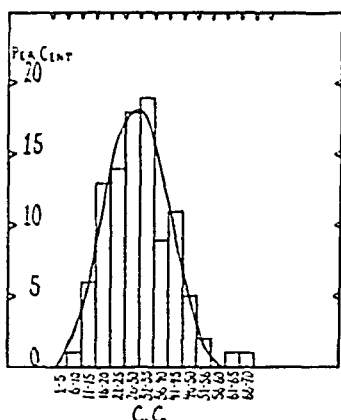


CHART 2

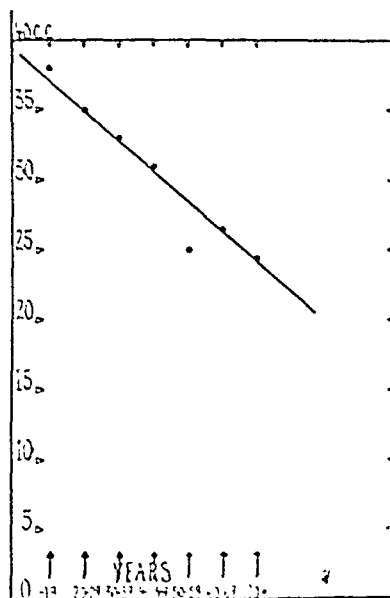


CHART 3

CHART 2. HISTOGRAM AND SMOOTHED CURVE OF DISTRIBUTION OF VOLUME OF SECRETION IN ENTIRE GROUP

CHART 3. AVERAGE MAXIMUM TEN-MINUTE SECRETORY VOLUME IN VARIOUS DECADES

secreted with advancing age becomes quite evident. The figures are graphically shown in chart 3. The explanation of this fact is still in doubt: whether there is a "physiological" deterioration of function, as people grow older, or whether the lessened secretion is the reflection of multiple focal injuries over a period of years, whereby secreting cells here and there have been damaged, can not be stated. The solution of the problem awaits histological study of stomachs

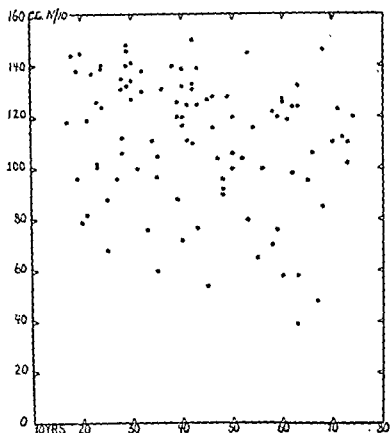


CHART 4

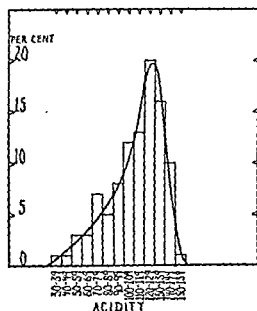


CHART 5

CHART 4. HIGHEST TITRATABLE (TOTAL) ACID IN EACH CASE PLOTTED AGAINST AGE OF SUBJECT

CHART 5. HISTOGRAM AND SMOOTHED CURVE OF DISTRIBUTION OF ACIDITY IN ENTIRE GROUP

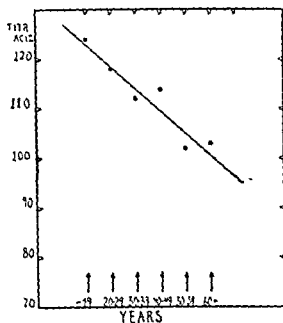


CHART 6

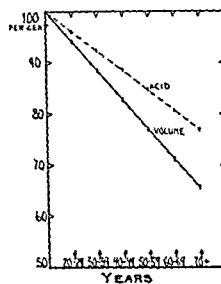


CHART 7

CHART 6. AVERAGE MAXIMUM ACIDITY IN VARIOUS DECADES
CHART 7. PERCENTAGE DECREASE IN VOLUME AND ACID WITH ADVANCING YEARS

ose function has been tested during life, and it is obviously difficult to obtain the necessary material where healthy people are concerned.

ACIDITY

In chart 4 the highest titratable acidity in each subject is shown. Here, as with volume of secretion, considerable variation is evident,

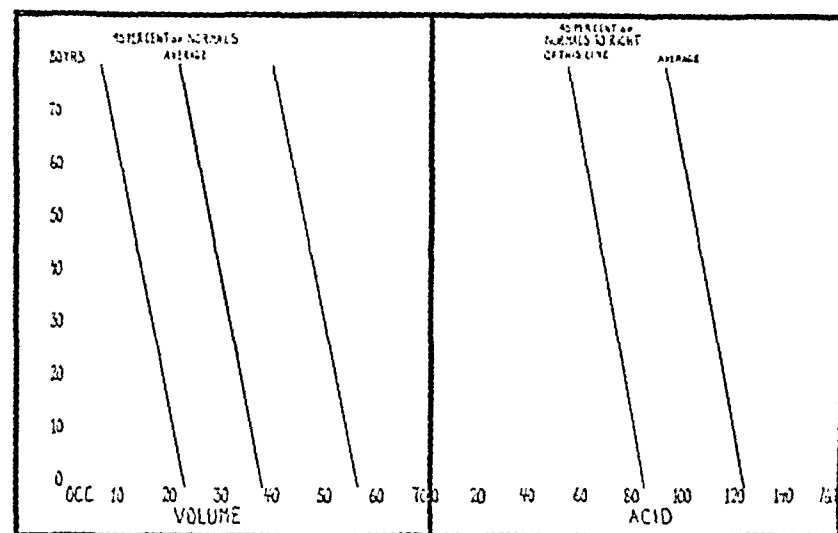


CHART 8. CHART FOR MAKING CLINICAL REPORT OF ACIDITY AND VOLUME OF GASTRIC SECRETION

From a statistical standpoint it would be preferable to have a series of vertical lines representing sigma or probable error, but in view of the small number of cases in each class this was not attempted. *It should also be noted that we have no data in the age periods below 15 years and that the average lines are therefore probably inaccurate for these periods. The chart should be used only to record observations made on adults.*

In the distribution curve (chart 5) is of different form. The frequency of the lower values gradually rises to be followed by a rapid increase above 90 to a peak, in the vicinity of 135, after which there is an abrupt descent; three fourths of the values lie above 100. There is evidently an upper level of concentration of acid in the vicinity of 135, which the normal stomach is incapable of surpassing, but under

powerful influence of histamine this value is often approached. definite lowering of the acid values with advancing age is seen when figures in various decades are averaged. The figures (chart 6) are not as striking as in the case of volume of secretion but are none the less definite. The explanation here also is not at hand. In chart the percentage decline in volume and acidity is shown simultaneously, the values in the youngest age period being taken as 100 per cent.

DISCUSSION

As we pointed out above, one of the main objects of these observations was to set standards with which findings in gastric disorders could properly be compared. Are there, for example, in cancer, in ulcer, in gastritis and in other diseases secretory findings which deviate in a characteristic way from the normal, so that practical diagnostic conclusions can be drawn? A glance at the distribution curves makes it evident that no exclusive findings are likely to be obtained; and, as is true of many similar problems in medicine, the matter resolves itself into a question of frequencies. In other words, if certain data are obtained from an instance of a gastric disorder, it will usually be difficult to say more than that such findings may be expected in a given percentage of normal people; the further the findings deviate from the normal distribution curve, the more likely they are to be indicative of abnormal conditions, and it is only by assembling large groups of cases that an approximate understanding of their clinical significance will be obtained. On this basis, as we have already pointed out elsewhere (1), useful practical information has been obtained bearing on the differentiation of ulcer and of cancer of the stomach. The following charts are suggested as the most illuminating means of reporting observations on gastric function in the Clinic (chart 8). If, in the case under consideration, the values for acid and volume are marked on such a chart, the relations of the findings to the normal may be visualized. The physician may then make his interpretation together with the clinical data as a whole.

In conclusion, it should be emphasized, that all of the present work has been done on adults. There is reason to believe that conditions may be quite different in children, and especially in infants.

NORMAL GASTRIC FUNCTION

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STUDIES ON THE PHYSIOLOGY OF THE PARATHYROID GLANDS

II. THE RELATION OF THE SERUM CALCIUM TO THE SERUM PHOSPHORUS AT DIFFERENT LEVELS OF PARATHYROID ACTIVITY.¹

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In 1922 Howland and Kramer (1) emphasized the fact that the deposition of calcium in the bones depends not alone on the calcium level in the blood, nor alone on the phosphorus level in the blood, but on the levels of both calcium and phosphorus. They refrained from any long physical-chemical discussion of ion products, etc., but contented themselves with multiplying the serum calcium in milligrams by the serum phosphorus in milligrams and arriving at the very useful clinical fact that, when this product was below 30 in young children or young rats, active rickets was present. When the product was above 40, rickets was either absent or healing had set in. Shipley, Kramer, and Howland (2) showed that when the product of $\text{Ca} \times \text{P}$ is 35 calcification is obtained in vitro, not only when the calcium is 10 mgm., and the phosphorus is 3.5 mgm., but also when the calcium is 5 mgm., and the phosphorus is 7 mgm. The original clinical observation of Howland and Kramer has stimulated many attempts from the laboratory to elucidate the physical-chemical laws which govern the calcium and phosphorus levels in the blood and the precipitation of these elements in the bone. Although much progress has been made, the subject is not as yet entirely clear. We believe that a return to the analysis of clinical data may throw further light on this subject. In this paper an analysis is made of the inter-relation of serum calcium

¹ The first publication of this series is 8 in the bibliography.

to serum phosphorus during varying degrees of hypo- and hyperparathyroidism.

There seems to be a growing conviction that normally the circulating fluid contains about all the calcium phosphate which that particular fluid system can hold at that particular time and that calcification is somewhat analogous to a precipitation of a calcium salt due to some local change in the environment favoring precipitation at the point of calcium deposit. The work of Robison, Soames, Kay, and Martland (3) (4) (5) (6) makes it appear likely that this local change at the point of precipitation is an increase in inorganic phosphates due to the hydrolysis of an organic phosphorus compound by a phosphatase. They find this phosphatase in growing bones and teeth, but not in ones before growth begins. With some such conception in mind one would perhaps be justified in dividing disorders of calcium metabolism (we might better say calcium phosphate metabolism) into three fundamental groups, viz.:

1. Conditions where the body fluids, as compared with normals, are deficient with respect to calcium phosphate. The serum calcium, the serum phosphorus, or both may be low—either actually or relatively to each other. As a result there is a failure of deposition of calcium phosphate in the osteoid matrix of bone and the pathological pictures of rickets or osteomalacia result.
2. Conditions where the body fluids contain more than the normal amount of calcium phosphate. This condition is less common but is met in certain destructive processes in the bones such as osteoclastic metastases and multiple myeloma and is also seen in ergosterol poisoning. It is associated with calcium phosphate deposits in tissues other than bones. In other words calcium deposits occur even where no precipitating factor is present.
3. Finally, conditions where the body fluids contain the normal amount of calcium phosphate, but where the relation of the calcium to the phosphorus is abnormal. Such, it will be seen, is our conception of the states existing in hypo- and hyperparathyroidism. If this concept is correct, an analysis of the relation of serum calcium to serum phosphorus here should give a clue as to what laws were governing their levels.

EXPERIMENTAL

Part I

We will first investigate the relation of serum calcium to serum phosphorus at various degrees of hypoparathyroidism. The data

TABLE 1

Data on patient with idiopathic hypoparathyroidism showing effect of parathormone medication on serum calcium and serum phosphorus

Day of experiment	Period of day*	Serum			Units of parathormone
		Ca	P	Ca × P	
		<i>mgm. per 100 cc.</i>	<i>mgm. per 100 cc.</i>		
2	2	5.0	10.7	53.5	
4	1	5.3	10.6	56.2	
4	2	5.3	10.4	55.1	
4	3	5.2	10.9	56.7	
6	1	5.4	10.9	58.9	50
6	2	6.2	9.5	58.9	
6	3	6.7	8.8	59.0	
7	1	7.1	8.4	59.6	50
7	2	8.5	7.8	65.9	
8	1	9.4	7.0	65.8	50
8	3	9.8	6.6	64.7	
9	1	9.8	6.0	58.8	50
9	2	10.5	5.4	56.7	
9	3	11.2	5.7	63.8	
10	1	10.1	5.7	57.6	
10	2	9.3	5.9	54.9	
10	3	8.8	6.5	57.2	
11	1	7.8	7.0	54.6	
11	2	7.8	7.6	59.3	
12	2	7.0	8.6	60.2	
13	1	7.0	8.8	61.6	
14	1	7.1	8.7	61.8	
15	1	7.7	8.7	67.0	
16	1	7.3	8.8	64.2	
18	1	7.7	8.6	66.2	
20	1	7.5	8.8	66.0	
21	1	7.4	8.6	63.6	
22	1	7.4	8.7	64.4	
Average.....				60.4	

* The days were divided into 3 eight-hour periods.

(table 1) consist of the 28 simultaneous serum calcium and serum phosphorus determinations taken in a boy with idiopathic hypoparathyroidism while the serum calcium was first being raised to normal by parathormone² and was later being allowed to fall as a result of cessation of parathormone medication (Albright and Ellsworth (8) — experi-

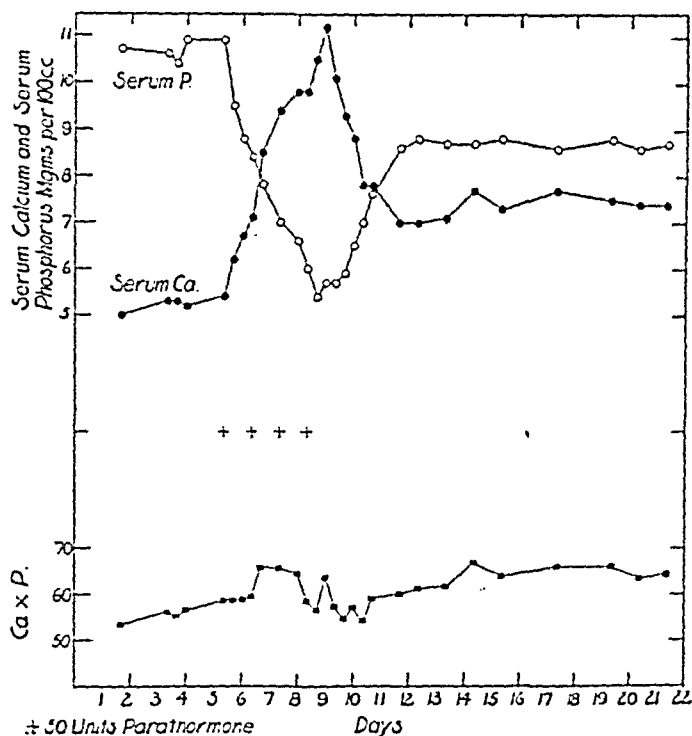


CHART 1. EFFECT OF PARATHORMONE ADMINISTRATION TO A PATIENT WITH IDIOPATHIC HYPOPARATHYROIDISM ON SERUM Ca, SERUM P, AND PRODUCT OF $Ca \times P$

ment 1). In chart 1 these data are given in graphic form. It will be noted that, as the serum calcium rises and falls, the serum phosphorus falls and rises. Further, it will be seen that the changes in the serum calcium and phosphorus are such that the product of $Ca \times P$ neither rises nor falls, but merely fluctuates about the base line. In other

² Preparation of parathyroid extract introduced by Collip (7) and supplied by Eli Lilly Company.

words the product of $\text{Ca} \times \text{P}$ tends to remain about constant. We have constructed chart 2 to show better what such a constancy implies. The curve in chart 2 shows to what degree the serum phosphorus must fall as the serum calcium rises if the average calcium phosphorus product (60.4) is to be maintained. The curve is a rectangular hyperbola, the general formula for which is $xy = C$. The practical importance

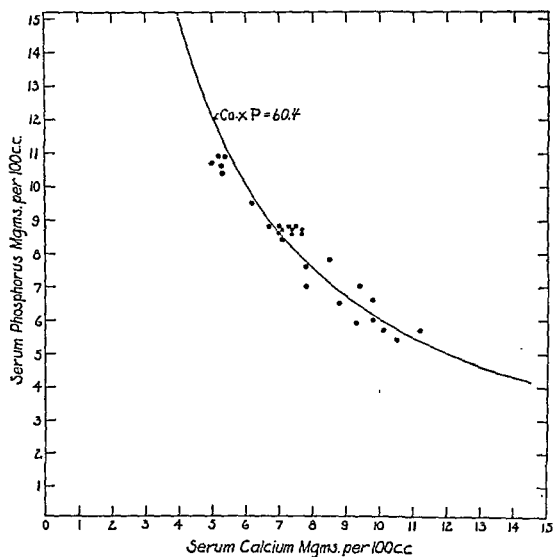


CHART 2. SHOWING HOW DATA FROM TABLE 1 FIT CURVE OF $\text{Ca} \times \text{P} = 60.4$

of the nature of the curve is that at low levels of calcium there must be a greater change in phosphorus for a given change in calcium than at high levels of calcium if the product is to remain constant. This has little significance with the figures now under consideration, but becomes important at the higher levels of calcium (*v. infra*). In chart 2 it will be noted that the individual determinations of serum calcium and serum phosphorus vary surprisingly little from such a theoretical curve.

TABLE 2

Data from eleven essentially normal patients who received parathormone medication and from two patients with hypoparathyroidism who received thyroid medication showing effect of medication on serum calcium and serum phosphorus

Group	Range of serum Ca	Number of determinations in group	Average serum Ca	Average serum P	Average Ca X P product	Condition
			mgm. per 100 cc.	mgm. per 100 cc.		
1	4.0-4.5	9	4.26	7.19	30.6	2 cases parathyroid tetany
2	4.5-5.0	20	4.79	6.50	31.1	
3	5.0-5.5	17	5.19	6.42	33.3	
4	5.5-6.0	19	5.79	5.76	33.4	
5	6.0-6.5	19	6.17	5.54	34.2	
6	6.5-7.0	8	6.68	5.60	37.4	
7	7.0-7.5	10	7.17	5.61	40.2	
8	7.5-8.0	9	7.69	5.63	43.3	
9	8.0-8.5	5	8.28	5.46	45.2	
10	8.5-9.0	7	8.70	5.18	45.1	
11	9.0-9.5	6	9.41	4.78	45.0	
12	9.5-10.0	4	9.81	4.61	45.2	
13	9.0-10.0	13	9.63	3.28	31.6	11 cases receiving parathormone
14	10.0-10.2	12	10.07	3.66	36.9	
15	10.2-10.4	14	10.28	3.39	34.8	
16	10.4-10.6	12	10.47	3.64	38.1	
17	10.6-10.8	13	10.66	3.65	38.9	
18	10.8-11.0	8	10.88	3.47	37.7	
19	11.0-11.3	12	11.12	3.43	38.1	
20	11.3-11.5	11	11.36	3.28	37.3	
21	11.5-12.0	15	11.75	3.10	36.4	
22	12.0-12.4	9	12.22	3.01	36.8	
23	12.4-12.9	9	12.66	2.79	35.3	
24	12.9-13.4	5	13.11	2.40	31.5	
25	13.4-14.0	3	13.65	3.84	52.4	
26	14.0-15.0	5	14.4	2.64	38.0	
27	15.0-16.0	5	15.52	2.87	44.5	
28	16.0-17.0	3	16.82	4.03	67.8	
Group A*		3	14.37	2.15	30.8	
Average of 13-24.....					36.1	

* This is a group of three determinations taken from groups 25 and 26 to show that a few points follow the curve as high as a serum calcium of 14.4.

The conclusion suggested from Part I is:

A. As the serum calcium varies between the level in the hypoparathyroid state and that in the normal state, or vice versa, the phosphorus varies in such a way that the product of $\text{Ca} \times \text{P}$ remains about constant.

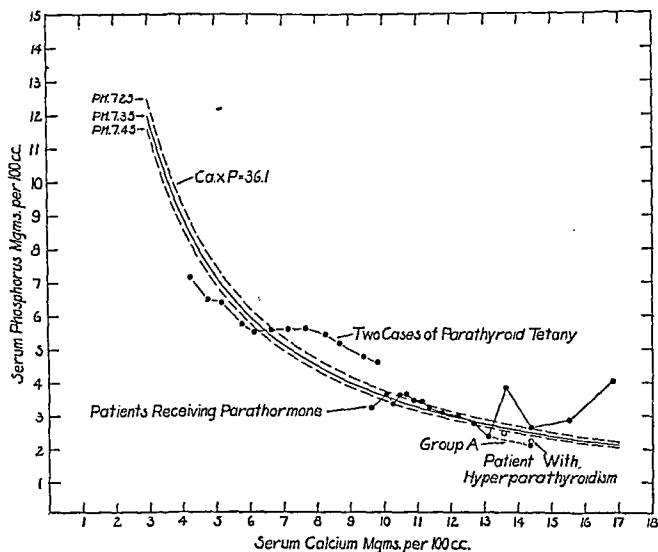


CHART 3. SHOWING HOW DATA FROM TABLES 2 AND 3 FIT CURVE OF $\text{Ca} \times \text{P} = 36.1$ AND INCLUDING (SEE BROKEN LINES) A GRAPHIC REPRESENTATION OF CORRECTION WHICH A $1/10$ OF A pH WOULD ENTAIL

Part II

We now turn to the data which deal with the relation of serum calcium to serum phosphorus at varying degrees of hyperparathyroidism. (See lower half of table 2—groups 13–28.) The data consist of 149 simultaneous determinations of serum calcium and phosphorus before, after, and during a parathormone administration to eleven essentially normal patients. The data are taken from the experiments reported by Albright, Bauer, Ropes, and Aub (9). The 149 blood determina-

tions have been arranged in order of increasing values of serum calcium and averaged by groups. The increment rise in calcium for each group has been made to vary in order that enough points to be averaged fall within any one increment. For each group the average values for the corresponding serum phosphorus and $\text{Ca} \times \text{P}$ product were also obtained. These data are shown graphically in chart 3 which is constructed similarly to chart 2. It will be seen that as the calcium rises from 9.6 to 13.1 the phosphorus falls sufficiently so that the product of $\text{Ca} \times \text{P}$ again remains about constant. The fall in the phosphorus, while definite, is small and corresponds to the fact that the slope of the theoretical curve is gradual at these higher ranges of calcium. Beyond calcium levels of about 13.5, the phosphorus suddenly rises abruptly from the smooth curve suggesting that some secondary phe-

TABLE 3

Data from patient with hyperparathyroidism showing relation of serum calcium to serum phosphorus

Group	Range of serum calcium	Number of determinations in group	Average serum Ca	Average serum P	Average $\text{Ca} \times \text{P}$ product	Condition
	<i>mgm. per 100 cc.</i>		<i>mgm. per 100 cc.</i>	<i>mgm. per 100 cc.</i>		
1	13.0-14.0	11	13.6	2.5	34.0	Hyperparathyroidism
2	14.0-15.3	12	14.4	2.25	32.4	Hyperparathyroidism

nomenon has entered in. Group A (see table 2 and chart 3) is a selected group of three determinations out of groups 26, 27, and 28, showing that a few bloods continue to follow the curve to a serum calcium level of 14 mgm. per 100 cc., suggesting that the critical point varies slightly in different individuals. This phenomenon of a sudden rise of the serum phosphorus at very high calcium levels will be discussed below.

In table 2 and chart 3 are recorded in a similar manner 133 blood determinations on two patients suffering from hypoparathyroidism (groups 1 to 12). Here we see much less correspondence with the theoretical curve. This is not surprising as many factors other than the degree of hypoparathyroidism were varied during the study. What some of these factors were, will be discussed below.

The conclusions suggested from Part II are:

B. As the serum calcium varies between the level in the normal state and that in the hyperparathyroid state, the phosphorus varies in such a way that the product of $\text{Ca} \times \text{P}$ remains about constant, except that,

TABLE 4

Data from patient with postoperative hypoparathyroidism showing effect of parathormone medication on serum calcium and serum phosphorus

Date	Time	Serum			Parathormone	Remarks
		Ca	P	$\text{Ca} \times \text{P}$		
		mgm. per 100 cc.	mgm. per 100 cc.		units	
1930						
March 15.....	7 a.m.	5.0	10.0	50.0	100	These first nine determinations were taken on the same day. The patient was fasting
	10 a.m.	5.1	10.0	51.0		
	11 a.m.	6.4	9.5	60.8		
	12 noon	5.9	9.8	57.2		
	1 p.m.	5.9	9.7	56.8		
	2 p.m.	7.0	8.3	58.0		
	3 p.m.	7.1	8.0	57.0		
	4 p.m.	6.6	7.8	51.2		
	5 p.m.	7.1	7.7	55.0		
May 19.....	8 a.m.	8.9	6.9	61.4	40 (daily) 40 (daily) 40 (daily) 40 (daily) 40 (daily) 60 (daily) 80 (daily) 80 (daily)	The last twelve determinations were taken while the patient was receiving a high calcium diet
May 22.....	8 a.m.	8.6	7.3	62.7		
May 26.....	8 a.m.	8.8	6.8	60.1		
May 27.....	8 a.m.	8.4	7.3	61.3		
May 28.....	8 a.m.	10.5	4.9	51.4		
May 29.....	8 a.m.	10.9	4.9	53.4		
May 31.....	8 a.m.	10.2	5.4	55.4		
June 2.....	8 a.m.	12.2	5.3	65.4		
June 3.....	8 a.m.	11.2	4.7	53.1		
June 5.....	8 a.m.	11.8	4.2	50.2		
June 6.....	8 a.m.	11.8	4.0	47.7		
June 7.....	8 a.m.	11.6	3.8	44.5		
Average.....				53.0		

C. At very high levels of serum calcium resulting from hyperparathyroidism, the serum phosphorus no longer falls but abruptly rises suggesting some secondary phenomenon.

D. Whereas low serum calcium levels in hypoparathyroidism are associated with much elevated serum phosphorus levels, high serum calcium values in hyperparathyroidism are associated, with only moderately

reduced serum phosphorus values, which is in entire agreement with the nature of the curve if $\text{Ca} \times \text{P}$ is to be a constant.

Part III

It is of interest to see what happens in hyperparathyroidism occurring pathologically rather than as a result of medication. In table 3

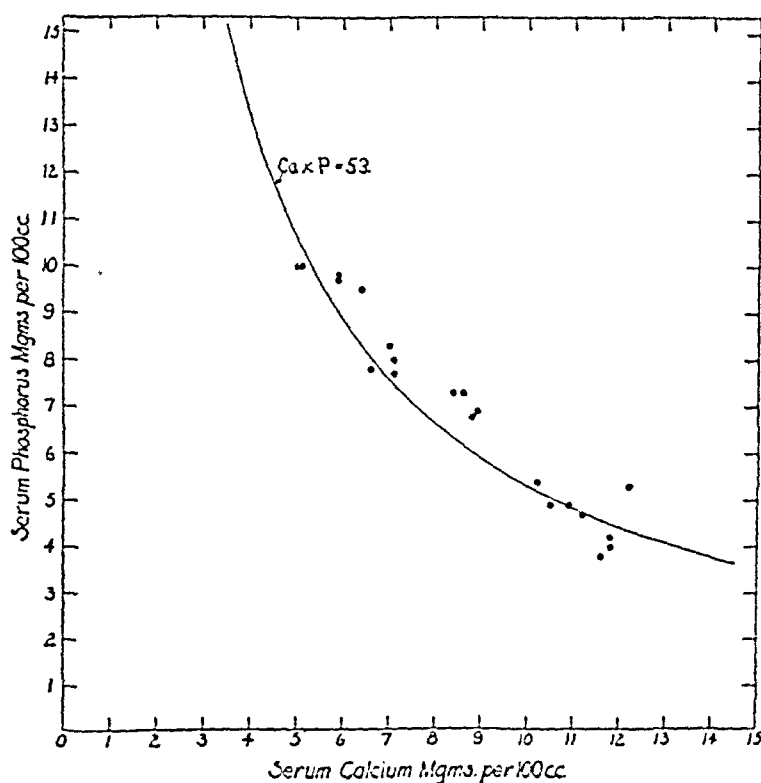


CHART 4. SHOWING HOW DATA FROM TABLE 4 FIT CURVE OF $\text{Ca} \times \text{P} = 53.0$

are given the data from 23 blood determinations on the patient suffering from hyperparathyroidism, reported by Bauer, Albright, and Aub (10). The values are represented in chart 3 by circles (q. v.). One sees that here again the values follow the theoretical curve.

Part IV

In table 4 and chart 4 are given the data from a patient with post-operative parathyroid tetany who with parathormone medication was

brought from the hypoparathyroid state through the normal state into the hyperparathyroid state. Whereas the other investigations recorded above were done on patients while on a low calcium diet, during the latter part of this study (see table 4) this patient was on a high calcium diet. Here, again, the product of $\text{Ca} \times \text{P}$ tended to remain constant, although there was some tendency for the phosphorus to fall relatively more than the calcium rose. If, as suggested by Albright and Ellsworth (8), parathormone acts by causing an increased excretion of phosphorus in the urine with a resulting lowering of the blood phosphorus, one would expect parathormone to lower the product of $\text{Ca} \times \text{P}$ temporarily until equilibrium was reestablished. It was their belief that equilibrium was as a rule so rapidly established that this lowering of the product was seldom observed. However, in the latter part of the present experiment, such a lowering did occur.

DISCUSSION

Obviously from data such as ours only graphs, showing the effect of the parathyroid hormone on the relation of serum calcium to serum phosphorus in actual clinical cases, can be constructed and the general type of curve outlined, but no definite conclusions as to the exact type of curve can be made. For instance, several years ago when some of these data were first assembled, we were influenced by the then recent work of Holt, LaMer, and Chown (11) in believing that the solubility product of tertiary calcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$) determines the amounts of calcium and phosphate in the blood. One had to assume that normally there was a marked but fairly constant degree of supersaturation. The equation to be satisfied (or rather exceeded by a constant amount) was:

$$[\text{Ca}^{+}]^3 \times [\text{PO}_4^{=}]^2 = K.$$

By making the further assumption that all the blood calcium was ionized, one obtained the simpler equation:

$$[\text{Ca}] \times [\text{P}]^{\frac{2}{3}} = \text{Constant}.$$

We found as a matter of fact that such a curve is sufficiently similar to $\text{Ca} \times \text{P} = K$ to fit our rough figures. One cannot escape the very

strong impression, however, that the general type of curve is such as would occur if phosphorus were varying with calcium in order to satisfy some solubility product constant,—be this that of secondary calcium phosphate, that of tertiary calcium phosphate, or that of some more complex salt. It would follow as a corollary to this, as suggested in the introduction, that the states of hypo- and hyperparathyroidism are both associated with an approximately normal degree of saturation of the blood with calcium phosphate, the only abnormality being a variation in the proportion of the calcium to the phosphate.

We have shown how our data tend to fit the curve $\text{Ca} \times \text{P} = \text{K}$ with the warning, again, that our data far from establish this curve. It may be more than coincidence that the same calcium phosphorus product which Howland and Kramer from *clinical* studies found valuable in determining whether or not rickets was healing we have found to fit the *clinical* findings in parathyroid dysfunctions. We are confronted with the question whether this product has any real significance or is merely a close approximation to some still obscure truth. We will not be able to answer this question, but a short survey of some of the pertinent facts may be helpful.

The suggestion of Shear and Kramer (12) that calcium and phosphate are precipitated as secondary calcium phosphate (CaHPO_4) has much in its favor to attract attention. The marked degree of supersaturation of the blood which one had to assume on the basis that they were precipitated as tertiary calcium phosphate no longer exists. Moreover, we are no longer dealing with a reaction of the third order which would make the rapid fluctuations in calcium and phosphorus resulting from parathyroid extract difficult to account for. When a solution is saturated with CaHPO_4

$$[\text{Ca}^{++}] \times [\text{HPO}_4^{--}] = K_{\text{S.P. Ca HPO}_4}$$

If all the calcium in such a solution is in the form of calcium ions and all the phosphate is in the form of ionizable phosphate salts, the simpler equation also holds:

$$\text{Ca} \times \text{P} = \text{Constant}$$

There is, however, very strong evidence that at least all the calcium is not ionized. Thus we would have no justification in using this second formula unless we could show that the error in so doing is constant. The constancy of our calcium-phosphorus products in that case would not be effected by a constant error.

What is the evidence for such an assumption? Salvesen (13) pointed out that

the amount of calcium in body fluids varies with the amount of protein. Cameron and Moorehouse (14) believed that the calcium of the spinal fluid, a practically protein free substance, represents the diffusible calcium of the plasma. It is not unlikely that the diffusible calcium and the ionizable calcium are approximately the same fractions. Shear, Washburn, and Kramer (15) (16) recently showed that, when protein free serum ("inorganic serum solution") is shaken with CaHPO_4 , much less CaHPO_4 is taken up than when serum containing protein is used. It would seem, therefore, that the amount of unionized calcium is roughly or perhaps exactly proportional to the amount of serum protein (if the phosphate is kept constant). But it has been repeatedly shown in this laboratory (unpublished data) that the level of serum protein is not effected by the parathyroid hormone. Since the unionized calcium is roughly proportional to the serum protein, it follows that this also would be little effected by the parathyroid hormone. The situation with the phosphates is less clear. Whereas normally the spinal fluid calcium is about one-half the serum calcium, likewise normally the spinal fluid phosphorus is about one-half the serum phosphorus (17). This by itself would suggest that the protein of the serum inactivates some phosphates as well as some calcium.

Furthermore, in hypoparathyroidism the difference between the serum calcium and the spinal fluid calcium diminishes, and the difference between the corresponding levels of phosphorus increases. These observations with respect to the relation of spinal fluid phosphorus to serum phosphorus have been under-emphasized in the literature. They strongly suggest that protein effects phosphates as well as calcium in body fluids. The actual figures obtained in a case of idiopathic hypoparathyroidism were as follows: with a serum calcium of 4.5 and a serum phosphorus of 6.2, there was a spinal fluid calcium of 4.5 and a spinal fluid phosphorus of 2.0; and later with a serum calcium of 6.7 and serum phosphorus of 4.4 there was a spinal fluid calcium of 4.7 and a spinal fluid phosphorus of 1.8. It would appear that in hypoparathyroidism the inactivated calcium decreases and that the inactivated phosphorus increases. Their product may be constant. In that case we would be dealing with a constant error due to inactivation of calcium ions and phosphate ions by proteins. One obtains the impression that protein by some process of interference alters the solubility product of CaHPO_4 . The error introduced by protein in using the quotient of serum calcium times serum phosphorus as an index may, consequently, be constant, in which case it could be disregarded. There is, therefore, some evidence other than empiricism to justify multiplying serum calcium by serum phosphorus.

There is some conflicting evidence as regards the effect of protein on inactivating phosphate ions. Grollman (18) showed that at normal ranges of calcium, 100 per cent of the serum phosphorus is filterable. Pincus, Peterson, and Kramer (19) found that the concentration of phosphorus in ultrafiltrates was the same as that in the original serum. Thus, if protein prevents some of the phosphates from exerting their full ionic values, it probably cannot be thought of as a strong union of protein and phosphates as has been considered in the case of calcium.

Even if one grants that the product of $\text{Ca} \times \text{P}$ tends to remain about constant at varying degrees of parathyroid activity, this could only be so if all other factors which effect this product were kept constant. We do not as yet know what all these factors may be. One can only hope to get consistent results if all possible factors such as diet, activity, exposure to ultraviolet light, etc., are kept as constant as possible, as in the experiments reported in Part I. The marked discrepancy, for instance, shown by the two cases of hypoparathyroidism discussed in Part II, may be due to any one of the many factors which were altered between the time when the calcium was low and the time when it was high. The elevation of the calcium in both of these cases was brought about by the thyroid hormone. The six cases of myxedema studied by Aub, Bauer, Heath, and Ropes (20) likewise showed an increase in the product of $\text{Ca} \times \text{P}$ under thyroid treatment as did the one case studied by Albright, Bauer, and Aub (21). Conversely, the case of Grave's disease in this latter study showed a reduction in the product of $\text{Ca} \times \text{P}$ coincident with treatment and reduction of metabolism. There is, then, some evidence that the thyroid hormone tends to increase the product of $\text{Ca} \times \text{P}$.

What are some of the other more important factors which may influence the product of $\text{Ca} \times \text{P}$? The serum protein requires no further discussion. There are three of special interest: (1) species of animal, (2) growth factor, and (3) hydrogen ion concentration.

In the rat the product of $\text{Ca} \times \text{P}$ is almost double that found in the human and the dog. If one assumes that this product is governed by physical-chemical laws there must be some modifying factor in the serum of the rat. The increase in the product is due to an increase in the phosphorus. Probably each species has its characteristic product depending on certain modifying variables. Likewise, to a lesser extent each individual in a species probably has its characteristic product.

It will be noted that the curve in chart 2 has a product of $\text{Ca} \times \text{P}$ of 60.4 while that in chart 3 has one of 36.1. The discrepancy is probably partly connected with the fact that chart 2 deals with a growing boy while chart 3 deals with adults. Hess and Lundagen (22) emphasized that during growth the serum phosphorus is elevated more than at other times. Shear and Kramer in their equilibration experiments

showed that the serum of normal young animals is more nearly saturated with respect to CaHPO_4 than the serum of older animals.

Whereas Shipley, Kramer, and Howland have demonstrated that, when the product of $\text{Ca} \times \text{P}$ is 35, calcification is obtained in vitro, Kramer, Shelling, and Orent (23) showed that when the pH is below 7.0, calcification is not obtained even with a product of 50. Shear and Kramer point out that at the reduced pH the product of $[\text{Ca}^{++}] \times [\text{HPO}_4^-]$ is reduced. This results because at a reduced pH the same amount of P produces fewer HPO_4^- ions. The degree of this change can be quantitatively determined from the equation taken from Shear, Washburn, and Kramer (15):

$$[\text{HPO}_4^-] = \frac{[\text{P}]}{1 + 10^{(pK_2' - \text{pH})}}$$

In chart 3, two broken line curves are constructed to show what difference a 1/10 of a point in pH makes. They are so constructed that their ion products equal that of the full line when allowance is made for the shift in pH. It will be seen that by this method of calculation a shift in the pH of a degree which one might find pathologically in the blood will account for only a small change in the product of $\text{Ca} \times \text{P}$. Thus, if there is an alkalosis in parathyroid tetany or an acidosis in hyperparathyroidism, it would not account for a marked shift in $\text{Ca} \times \text{P}$ product. Wilson, Stearns and Thurlow (24) from a study of the dissociation of oxyhemoglobin following parathyroid tetany in dogs, concluded that there was a tendency to alkalosis, in hypoparathyroidism. McCann (25) found a rise in blood CO_2 in two dogs suffering from parathyroid tetany. Hastings and Murray (26) were unable to substantiate these findings. In one of our cases the CO_2 combining power of the serum which was done at a time when the serum calcium was low (4.5 mgm.), was found to be high (75 volumes per cent). Brehme and György (28) found a slight lowering of pH as a result of administering parathormone.

As further evidence of the slight effect of changes in pH on the calcium-phosphorus product, table 5, is included. Here the effect of an ammonium chloride acidosis on a patient with idiopathic parathyroid tetany is illustrated. Although the acidosis caused a rise in

serum calcium as so often is the case, the calcium-phosphorus product was only slightly raised.

There remains to be discussed the abrupt rise in serum phosphorus at the very high levels of serum calcium. This is in entire agreement with the work of Collip (7). He has emphasized that after the serum calcium has risen to a certain critical point, there is a shut-down in kidney function with a rapid rise in serum phosphorus, nonprotein nitrogen, etc. The work of Grollman (18) may be significant here. He showed that whereas normally 100 per cent of serum phosphate is filtrable, at high levels of calcium this is no longer true. He further showed that injection of parathormone into a dog produced a state

TABLE 5

Data from patient with hypoparathyroidism showing effect of the production of an acidosis on serum calcium and serum phosphorus

Date	Serum						
	Ca	P	Ca × P	CO ₂	Cl	Protein	Total/ base
1927	mgm. per 100 cc.	mgm. per 100 cc.		volumes per cent	mgm. per 100 cc.	per cent	cc. of N/10
May 11.....	4.5	6.2	27.9	75.3	550	6.7	156
May 18.....	4.6	6.7	30.8	52.1	614		150
May 25.....	5.2	5.9	30.7	54.6	614	7.7	156
June 2.....	6.7	4.4	29.5	44.0	632	8.0	153
June 14.....	5.3	5.3	28.1	85.5	540	6.9	150

(calcium = 17.9) where only 63 per cent of the inorganic phosphorus was filtrable. This rather suggests that, at high levels of calcium, it may be difficult to excrete the inorganic phosphorus through the glomeruli. A diminution in phosphorus excretion in the urine at high levels of calcium was apparent in the patients receiving large doses of parathormone ((9), observation XXVIII, p. 159). As a result phosphorus may pile up in the blood. When this phenomenon does occur, it would appear that there must be a marked supersaturation of the blood with CaHPO_4 . This is supported by the work of Hueper (27), which has been repeated by one of us (F. A.), who found calcification in the thyroid glands, mucous membrane of stomach, lungs, and kidneys of dogs dying from parathormone overdosage. We would

emphasize that this whole phenomenon is a secondary one, a complication of kidney shut-down as it were, and has probably nothing to do with the fundamental action of parathormone. It leads to a disorder of calcium and phosphorus metabolism where the body fluids contain more than the normal amount of calcium phosphate and thus may be compared with the ergosterol poisoning where similar pathological calcifications are produced.

SUMMARY

1. From an analysis of 354 simultaneous determinations of serum calcium and serum inorganic phosphorus in patients at varying degrees of hypo- and hyperparathyroidism, it would appear that one rises as the other falls to such a degree that their product remains roughly constant.

2. It is pointed out that such a constancy implies a *slight* reduction of phosphorus at high levels of calcium but a *marked* increase of phosphorus at low levels of calcium.

3. The question is raised whether this constancy represents the direct expression of a law or whether it is merely a close approximation to some law. In this connection it is pointed out that if calcium and phosphate are precipitated in the bones as secondary calcium phosphate and if the effect introduced by serum protein can be shown to be constant there may be chemical support to such a constancy.

4. The effects of serum protein, the thyroid hormone, the species of organism, the phenomenon of growth and pH on the product of $\text{Ca} \times \text{P}$ are discussed.

5. It is believed that dysfunctions of the parathyroid glands belong to that group of calcium disorders in which the body fluids contain a normal amount of calcium phosphate but in which the relation of the calcium to the phosphate is abnormal.

6. However, at very high levels of calcium a secondary phenomenon occurs. Phosphorus is no longer excreted in the urine and serum phosphorus abruptly rises. This results in a disorder of calcium metabolism where the body fluids contain an increased amount of calcium phosphate and leads to calcium phosphate precipitation in tissues other than bone.

7. The data give no clue as to whether with parathyroid administra-

tion the serum calcium rises because the serum phosphorus falls, or whether the serum phosphorus falls because the serum calcium rises. Based on this data alone one supposition is as logical as the other.

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STUDIES ON THE VELOCITY OF BLOOD FLOW

XV. THE VELOCITY OF BLOOD FLOW AND OTHER ASPECTS OF THE CIRCULATION IN PATIENTS WITH "PRIMARY" AND SECONDARY ANEMIA AND IN TWO PATIENTS WITH POLYCYTHEMIA VERA¹

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Claude Bernard (1) stated that ". . . the various systems of the body have protective functions to place in reserve the substances essential to life and to maintain uninterruptedly the humidity, warmth and other conditions indispensable to vital activity." The purpose of the present investigation is to study the protective function of the velocity of blood flow in compensating for deficient oxygen carrying capacity of the blood. When the oxygen carrying capacity of the blood is diminished two mechanisms are available to maintain an adequate supply of oxygen to the tissues (22). These mechanisms may act singly or together. The first of these mechanisms consists in relatively more complete abstraction of oxygen from the blood as it passes through the capillaries (16). Normally, 100 cc. of arterial blood contain approximately 18 cc. of oxygen. Under normal basal conditions, only about 5.5 cc. are removed from the blood as it passes through the capillaries. The remaining 12.5 cc. may be regarded as reserve oxygen which can be called upon during exercise or other unusual states to prevent asphyxia of the tissues. The anemic patient in relying on this mechanism of more complete oxygen abstraction, diminishes his reserve oxygen and sacrifices this factor of safety, the degree of sacrifice depending upon the severity of the anemia.

The second mechanism which may compensate for a deficient concentration of hemoglobin consists of an increase in blood flow. If

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the concentration of hemoglobin is 50 per cent of normal, the blood flow may be doubled. Under such circumstances, the amount of oxygen withdrawn from each cubic centimeter of blood would be one half the normal, but the total amount of oxygen given off to the tissues would be unchanged and the total reserve oxygen would be undiminished. The heart, however, would be required to expend an abnormal amount of energy, and the circulatory reserve would be encroached upon. The extent to which the pulmonary blood flow is accelerated in the presence of anemia has not previously been studied; the following investigation was therefore undertaken in order to gain further knowledge regarding the degree to which increased rate of blood flow compensates for deficient concentration of hemoglobin.

METHODS USED

Preceding studies (2, 3, 4, 5) have shown the feasibility of intravenous injection of radium C for measurement of the velocity of blood flow from the arm to the heart (arm to heart time) and of the velocity of pulmonary blood flow (pulmonary circulation time). The method appeared particularly suited to the study of the circulatory adjustment to anemia because, in contrast to circulatory minute volume estimations, the measurements are more direct and do not involve elaborate estimations of the CO_2 dissociation curves in each patient as is necessary in the carbon dioxide methods. In this research, all measurements were obtained under basal metabolic conditions. The pulse rate was counted several times before and after each test. The venous pressure was estimated according to the direct venipuncture method of Moritz and Tabora. The vital capacity of all patients was measured by means of a Collins spirometer. The hemoglobin concentration of the peripheral blood was measured by the Newcomer method. The blood plasma volume was estimated by the brilliant vital red method used by Thompson (30). In several patients with pernicious anemia observations were made when the hemoglobin concentration of the blood was low and later when, after treatment with liver extract (23), the blood more nearly approached normal.

RESULTS AND DISCUSSION

Thirty-two complete series of measurements were made in twenty-nine subjects with pernicious anemia, with secondary anemia and carcinoma, and with secondary anemia due to causes other than carcinoma (table 1). All patients were free from evidence of congestive heart failure. The patients have been divided into several groups according to the etiology of the anemia in order to learn whether the circulatory adjustment differs according to the underlying pathological condition.

The circulatory adjustment in patients with "primary" anemia and anemia secondary to diseases other than carcinoma

To clarify the relation between certain important findings, the relation between the hemoglobin and the pulse rate (fig. 1), between the pulse rate and the velocity of pulmonary blood flow (fig. 2), and the relation between the velocity of pulmonary blood flow and the hemoglobin concentration of the blood (fig. 3) were plotted in patients with primary anemia and in patients with secondary anemia not due to carcinoma. The results show that, while there are considerable variations, the velocity of blood flow through the lungs in these patients generally tends to increase in proportion to the degree of anemia. The variations may well be due to small differences in the basal metabolic rate, some investigators having found normal values, others an increase (15). Previous studies have indeed shown that the pulmonary circulation time may be affected by the metabolic rate (6, 7). The variations may also be due in part to the fact that different individuals with anemia probably rely in varying degree on the abstraction of a greater percentage of oxygen from the capillary blood.

These alterations in the velocity of blood flow are in accord with observations on the minute volume output of the heart in anemia. Plesch (26), by an indirect method, found that the total volume output was always increased roughly in proportion to the severity of the anemia. Liljestrand and Stenström (20) likewise observed a rise in the minute volume output of the heart but believed that the oxygen unsaturation of the venous blood in anemia was greater and that the increase in the minute volume output was less than that found by

TABLE 1

Measurements of the pulmonary circulation time and related functions in patients with anemia and with polycythemia vera

Subject	Age	Vital capacity per square meter	Pulse rate	Hemo globin	Red blood cells	Plasma volume per kilogram	Blood volume per kilogram	Arterial pressure			Circulation time		Diagnosis
								Sys- tolic	Diastolic	Mean	Arm to heart	Pulmonary	
Group A. Patients with secondary anemia													
		cc.		percent	millions per cu. mm.	cc.	cc.	mm. Hg	mm. Hg	mm. Hg	seconds	velocity per cent. of normal	
C. H.	27	1,320	122	33	1.85	58.7	68.0	90	65	77	3.0	6.5	Miscarriage
H. B.	21	1,940	96	30	1.42			100	60	80	6.0	6.5	Miscarriage
C. M.	30	1,480	108	48	3.10			100	60	80	3.5	7.0	Miscarriage
J. S.	50	2,260	74		2.47	53.7	69.6	148	80	114	6.5	7.0	Duodenal ulcer
P. L.	27	2,200	102	54	3.58	49.5	70.0	110	80	95	5.0	8.0	Duodenal ulcer
H. W.	26	2,180	102	37	1.80	52.0	64.5	100	60	80	4.0	8.0	Miscarriage
C. M.	34	2,140	57	38	2.12	51.8	60.0	90	50	70	6.5	9.0	Peptic ulcer
J. C.	42	2,320	60	46	2.45	52.3	65.9	100	65	82	12.0	10.0	Duodenal ulcer
O. C.	58	2,290	81	62	2.85			120	80	100	12.0	10.0	Peptic ulcer
F. T.	65	1,170	75	37	1.67	61.7	74.6	155	85	120	8.0	10.5	Chronic nephritis
C. D.	72	1,830	72	60	2.78	42.5	59.6	130	80	105	3.0	14.0	Scurvy; arteriosclerosis
T. K.	53	1,900	62	70	3.75	53.0	83.7	110	70	90	24.5	14.5	Duodenal ulcer
Group B. Patients with pernicious anemia													
M. A.	54	1,150	82	30	1.86	58.2	75.0	110	80	95	2.5	7.0	Combined system disease
M. A.	54	1,620	66	64	3.54	37.3	56.2	100	60	80	8.0	8.0	
D. T.	55		70	36	2.02	75.3	90.3	110	80	95	6.5	8.5	
F. D.	43	1,890	76	60	2.62	61.9	89.9	116	80	98	12.0	9.0	
S. B.	53	1,160	68	69	2.74	48.7	70.5	120	76	98	8.0	9.0	
F. H.	65	1,010	87	27	0.86			118	52	85	7.0	9.0	
A. H.	56	1,800	86	39	1.52	54.3	65.7	100	55	77	8.0	10.0	
D. T.	55	1,570	65	64	2.86	49.1	65.5	130	70	100	9.0	10.0	
W. M.	54	2,230	63	55	2.0	59.5	80.0	110	60	85	11.0	13.0	
A. H.	56	2,030	72	70	3.2			100	60	80	17.0	17.0	

Group C. Patients with carcinoma

M. E.	67	1,550	96	24	1.78	45.6	52.9	112	70	91	4.5	7.0	154	Carcinoma of pylorus
A. W.	54	1,830	70	67	4.06	55.6	90.6	116	84	100	5.0	8.0	135	Carcinoma of stomach
B. G.	70	1,050	100	59	3.60	49.8	72.1	110	60	85	5.0	11.0	98	Carcinoma of stomach
J. M.	53	1,730	84	28	1.74	54.5	63.9	140	80	110	12.5	12.0	90	Carcinoma of stomach
J. S.	65	1,680	72	55	3.70			96	58	77	7.0	12.0	90	Carcinoma of pylorus
J. H.	62	1,174	105	80	3.97		89.8	90	68	89	5.5	12.0	77	Carcinoma of stomach
P. M.	46	1,970	108	58	3.59	61.9	74.2	100	50	70	10.5	15.0	72	Carcinoma of colon
M. M.	65	1,270	84	60		51.6			60	80	8.5	16.0	67.5	Carcinoma of sigmoid
M. C.	76	1,150	82	31	2.70	64.1	80.0	90	45	67	4.0	16.5	65	Carcinoma of colon
P. H.	40	1,080	84	57	3.30	64.7	96.0	95	50	72	13.0	24.0	45	Carcinoma of esophagus

Group D. Patients with polycythemia vera

E. M.	42	2,780	86	147	10.56	45.8	175.0	120	90	105	17.0	17.0		Auricular fibrillation; pericarditis
E. M.	42	2,850	78	147	10.56	45.8	175.0	130	92	111	10.0	17.0		
P. S.	54	1,160	80	156	8.99	45.6	149.0	106	78	92	21.0	26.0		

Plesch. The observations of Richards and Strauss (27) were in entire agreement with the findings of Liljestrand and Stenström (20).

Certain investigators have inferred the general state of the circulation in anemia by studying the difference in oxygen content of the arterial and venous bloods of the arm. Morawitz and Röhmer (24) observed a relatively greater loss of oxygen during anemia than nor-

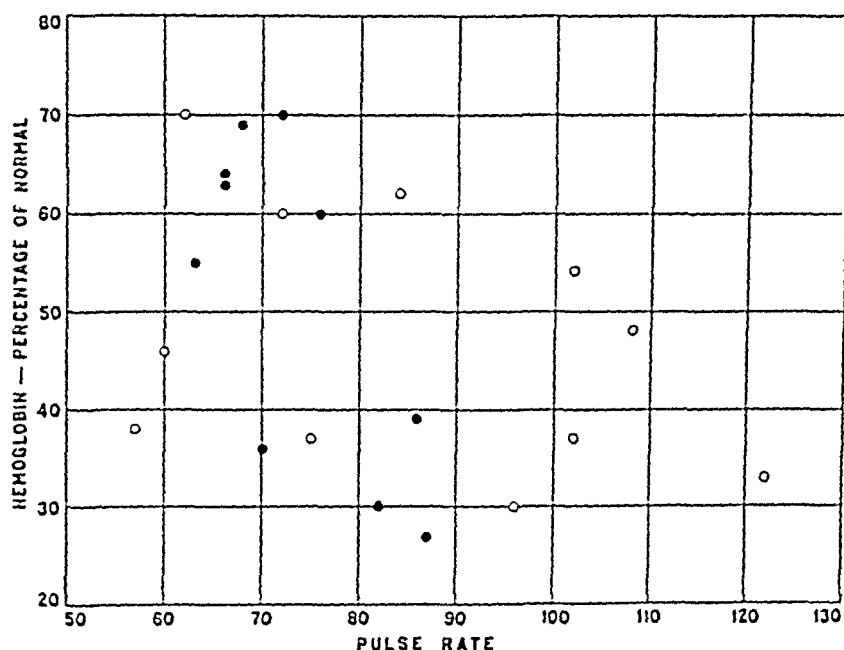


FIG. 1. RELATION BETWEEN HEMOGLOBIN CONCENTRATION (PERCENTAGE OF NORMAL) AND PULSE RATE IN PATIENTS WITH "PRIMARY" ANEMIA AND ANEMIA SECONDARY TO DISEASES OTHER THAN CARCINOMA

The solid dots refer to measurements in patients with "primary" anemia, the circles, to measurements in patients with anemia secondary to diseases other than carcinoma.

mal indicating that a certain degree of compensation in anemia is attained by increased oxygen unsaturation. They considered, that an increase in blood flow was, however, of still greater importance. Lundsgaard (21), as a result of his studies of the peripheral blood, concluded, "The results seem to show that the resting organism does not increase its circulation until all the reserve oxygen is used. This

means that the resting anemic organism does not need or use any compensation for its anemia until the hemoglobin has sunk below 30 per cent. Below that value the organism increases the blood flow in order to secure to the tissues the normal amount of oxygen."

It is questionable, however, whether results gained by the study of the blood flow through the arm can rightly be used as an index of the

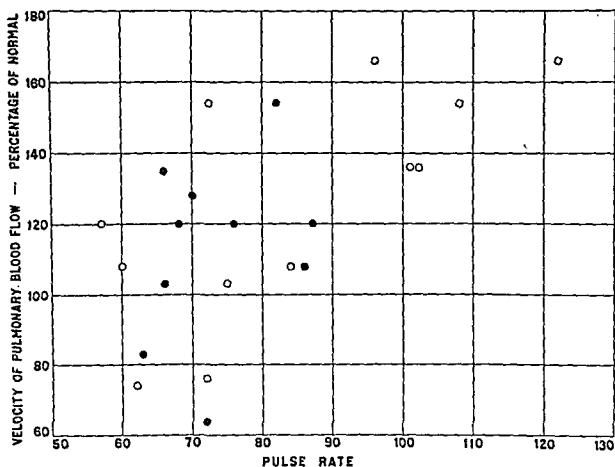


FIG. 2. RELATION BETWEEN THE PULSE RATE AND THE VELOCITY OF PULMONARY BLOOD FLOW IN PATIENTS WITH "PRIMARY" ANEMIA AND ANEMIA SECONDARY TO DISEASES OTHER THAN CARCINOMA

The solid dots refer to measurements in patients with "primary" anemia, the circles, to measurements in patients with anemia secondary to diseases other than carcinoma.

general circulatory adjustment of the body. G. N. Stewart (29) was evidently of similar opinion. He found that the volume of blood flow in the arms was diminished in anemic patients but stated that this might be due to peripheral vasoconstriction allowing the volume flowing through other parts of the body to be markedly increased.

It should be noted that the vital capacity of the lungs in our patients was moderately reduced in the absence of any signs of congestive

heart failure. Some patients complained of weakness and fatigue but these factors, according to Peabody and Sturgis (25), are not important in causing a reduction of the vital capacity of the lungs. We are unable to explain this lowering in the vital capacity, though it may well be related to the presence of an increased amount of blood in the lungs (14, 6), coincident with an increased rate of blood flow.

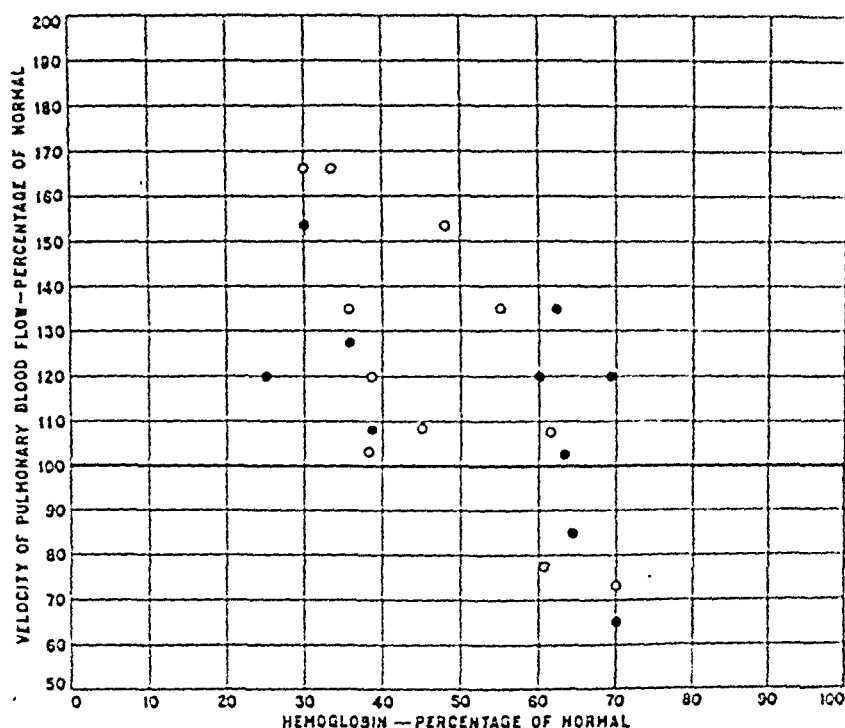


FIG. 3. RELATION BETWEEN THE VELOCITY OF PULMONARY BLOOD FLOW AND THE HEMOGLOBIN CONCENTRATION OF THE BLOOD

The solid dots refer to measurements in patients with "primary" anemia, the circles, to measurements in patients with anemia secondary to diseases other than carcinoma.

The mean plasma volume per kilogram of body weight was within the range of normal, again indicating the constancy of this characteristic of the circulation (8). The mean blood volume per kilogram was moderately decreased.

In previous communications the response of the circulation to wide

variations in basal metabolic rate in myxedema and thyrotoxicosis was studied (6, 7). The pulse rate was related more closely to changes in pulmonary blood velocity than to changes in the basal metabolic rate. Similarly, in the present study the pulse rate is more closely related to changes in velocity of the pulmonary circulation than to variations in the degree of anemia. This result is in accord with expectation for the pulse rate and velocity of blood flow are both characteristics of the general circulatory adjustment and as such are more closely related physiologically to each other than to change in the oxygen carrying capacity of the blood.

During muscular exercise in normal individuals the minute volume output of the heart rises as a linear function of the oxygen absorption, but at the same time the oxygen abstraction is on the whole more complete, though the type of work may to some extent affect the relative degrees to which these two mechanisms are employed (11, 12). In thyrotoxicosis, on the other hand, the compensation is almost entirely circulatory for the increased oxygen demands of the body are usually supplied without diminishing the oxygen tension of the mixed venous blood (19, 28). In anemia the greater the diminution in hemoglobin, the greater the extent, even at rest, to which the blood supply must be increased to supply adequate amounts of oxygen to the tissues. Under such circumstances, any muscular exercise places a relatively great burden on the cardiovascular system. This doubtless is an important factor in the frequent presence of dyspnea in patients with severe anemia, such as pernicious anemia. The clinical observations of Herrick (17), Bullrich (9), and Coombs (13) that anemic patients with angina pectoris may be relieved of the attacks of pain by improving the condition of the blood is readily understood on the basis of these considerations. The heart in such patients may be damaged to so slight an extent that it is able to maintain an adequate blood flow provided that the oxygen carrying capacity of the blood is normal. In the presence of anemia, however, the increased amount of work necessary to compensate for this condition cannot readily be accomplished, particularly since the blood supply to the heart is affected in common with that of the rest of the body.

The circulatory adjustment to secondary anemia in patients with carcinoma

Patients with anemia secondary to carcinoma were studied as a separate group because clinical experience suggests that the circulation is frequently affected adversely in this condition. Symptoms such as dyspnea, signs of congestive failure, peripheral edema, weakness and cyanosis are frequently more pronounced than one would expect on the basis of the degree of the anemia, malnutrition or toxicity (10). The findings in the group of patients studied are in accord with clinical experience. The average concentration of hemoglobin was practically the same in patients with carcinoma and with pernicious anemia, but the average velocity of pulmonary blood flow was reduced to 89 per cent of normal when carcinoma was present but was raised to 113 per cent in patients with pernicious anemia.

The pulmonary circulation time and related functions of the circulation in two patients with polycythemia vera

Since the velocity of blood flow is increased in patients with a diminished concentration of hemoglobin, it was thought to be of interest to learn whether the circulation is slow in patients with abnormally great concentrations of hemoglobin. In contrast to the unusually rapid blood flow found in the former group, two patients with polycythemia vera showed definite retardation of the blood flow below the average of normal. In one patient the extent to which slowing was related to the increased amount of hemoglobin cannot be accurately stated, for, although the patient showed no signs of congestive failure at the time of the test, fibrillation of the auricles was present. We have observed, however, that in the absence of signs of circulatory failure the blood velocity (4) may be normal, even in the presence of this abnormal mechanism. This slowing of the blood flow in polycythemia vera corresponds in degree to the diminished minute volume output of the heart observed by Liljestrand and Stenström (20). The increase in blood volume is due to the increased number of red blood cells, the plasma volume per kilogram of body weight being greatly diminished below the average of normal (18). The characteristics of the blood are the reverse of those present in anemia and the circulatory adjustment is correspondingly altered.

These findings illustrate anew the close interrelationships among apparently diverse functions of the body; in this case, among the respiratory, circulatory and metabolic systems. In a former study of the velocity of blood flow and related functions of the circulation in pulmonary emphysema evidence was presented which suggested that the circulation was accelerated to compensate for the defect in "external respiration" (31). In this communication evidence is presented which indicates that, similarly, in patients with anemia, the circulation is accelerated to compensate for failure of the "internal respiration."

SUMMARY AND CONCLUSIONS

1. The degree to which increased blood flow compensates for a deficient concentration of hemoglobin was studied in patients with pernicious anemia, with secondary anemia and carcinoma, and with secondary anemia due to causes other than carcinoma.

2. Thirty-two complete series of measurements of the vital capacity of the lungs, the blood plasma volume, the pulse rate, the arterial and venous blood pressures, and the velocity of blood flow through the lungs and from the arm to the heart were made in twenty-nine subjects.

3. In patients with "primary" anemia or with secondary anemia not due to carcinoma, the velocity of blood flow through the lungs tended to increase in proportion to the degree of anemia.

4. The increased burden on the cardiovascular system is accentuated by exertion and serves to explain the frequent presence of dyspnea in severely anemic patients and also the alleviation of angina pectoris in such patients after improvement in the condition of the blood.

5. The average concentration of hemoglobin was practically the same in patients with anemia due to carcinoma as in patients with pernicious anemia but the velocity of pulmonary blood flow in the former group was relatively slower. This serves to explain why symptoms such as dyspnea, signs of congestive failure, peripheral edema, weakness and cyanosis are frequently more pronounced in patients with carcinoma than one would expect on the basis simply of anemia, malnutrition or toxicity.

6. In contrast to the unusually rapid blood flow in patients with anemia, two subjects with polycythemia vera showed definite retarda-

tion of the blood flow below the average normal; the characteristics of the blood in this condition are the reverse of those present in anemia and the circulatory adjustment is correspondingly altered.

7. In a previous study of the velocity of blood flow and related functions of the circulation in pulmonary emphysema evidence was presented which suggested that the circulation was accelerated to compensate for the defect in "external respiration." In this communication evidence is presented which indicates that, similarly, in patients with anemia, the circulation is accelerated to compensate for failure of the "internal respiration."

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STUDIES IN CONGESTIVE HEART FAILURE

VIII. THE EFFECT OF THE ADMINISTRATION OF DIBASIC POTASSIUM PHOSPHATE ON THE POTASSIUM CONTENT OF CERTAIN TISSUES¹

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Previous investigations have shown that the potassium contents of both the skeletal and the cardiac muscle of patients dying of congestive heart failure were diminished (Harrison, Pilcher and Ewing, 1930). Analyses of tissue obtained by biopsy indicated that edema was probably the cause of the loss of potassium from the skeletal muscle (Pilcher, Calhoun, Cullen and Harrison, 1930). Studies on hearts of subjects dying under various conditions led to the belief that overwork was probably responsible for the loss of potassium from the cardiac muscle (Calhoun, Cullen, Clarke and Harrison, 1930). In order to determine whether or not this chemical change is irreversible the observations described in this paper were carried out.

METHOD

According to the technique described in our previous publications tissues were obtained at the postmortem table and were analyzed for potassium and for total solids. Specimens were obtained from the heart and from the gastrocnemius muscle in all the cases. In some instances portions of the liver and kidney were also analyzed.

The potassium content and total solids were determined in tissue from three classes of patients, (1) patients with heart disease who had never received potassium, (2) patients with heart disease who had

¹ Aided by a grant from the National Research Council.

received potassium, (3) a control group of patients without heart disease and whose hearts appeared normal at autopsy.

In the series of patients who died of heart disease there were fourteen individuals with clinical and pathological evidence of failure of both ventricles, i.e., both systemic and pulmonary congestion. All of these subjects had had some pitting edema in the past, and in many edema had been massive. Seven of these subjects had been treated in the usual way and had not received potassium salts. The other seven subjects had had the usual treatment and had also taken potassium dibasic phosphate by mouth for varying periods of time. (This salt of potassium was chosen because Laszlo (1928) found decreased phosphate content of the skeletal and cardiac muscle in patients with cardiac disease.) Five of them received this salt in doses of six grams daily. One patient was given twice as much. The seventh individual took potassium iodide for six months and potassium dibasic phosphate for two weeks. The length of time during which potassium salts were administered to each subject is given in the tables.

Complete data concerning the clinical state of these patients who were given potassium phosphate will be presented in a later paper.

RESULTS

The values for total solids and for potassium obtained in this study are presented in tables 1 to 5, each table reporting the value of all three groups of cases for one type of tissue. The average values for the entire series are summarized in table 6. The data for potassium in dry tissue is presented graphically in figure 1. In the tables, the potassium values are given for both "dry" and "wet" muscle, but figure 1 presents only the values for "dry" muscle since a similar chart based on "wet" muscle gives entirely comparable results. In the discussion of potassium contents of the various tissues the percentage for "dry" muscle is used throughout.

TABLE 1
The solids and potassium content of skeletal muscle

Subject	Chief diagnosis	Solids	Potassium in dry tissue	Potassium in wet tissue	Daily dose of K_2HPO_4	Duration of administration of potassium	Remarks
		per cent	per cent	per cent	grams	weeks	
W. S.	Acute peritonitis	24.3	1.36	0.337	0	0	No cardiac disease
E. A.	Lymphosarcoma	22.2	1.39	0.309	0	0	
R. D.	Brain tumor	27.1	1.14	0.308	0	0	
O. F.	Pulmonary tuberculosis	25.6	1.50	0.387	0	0	
J. F.	Syphilitic myocarditis	17.9	0.87	0.156	0	0	Cardiac failure; never received potassium dibasic phosphate
J. J.	Syphilitic aortic insufficiency	19.8	0.79	0.157	0	0	
J. B.	Chronic nephritic hypertension	25.1	1.18	0.297	0	0	
E. R.	Cardiac hypertrophy	17.7	0.78	0.138	0	0	
J. R.	Hypertension	18.5	0.86	0.155	0	0	
F. H.	Hypertension	26.9	0.99	0.266	0	0	
E. H.	Mitral stenosis	16.3	1.39	0.227	0	0	
G. H.	Hypertension	19.5	1.48	0.288	12	2.5	Cardiac failure; received potassium dibasic phosphate
J. C.	Syphilitic aortic insufficiency	21.9	0.81	0.177	6	5.0	
H. M.	Mitral stenosis	17.9	1.65	0.297	6	6.3	
B. P.	Hypertension	30.2	1.20	0.363	6	28.7	
A. D.	Hypertension	27.0	1.20	0.324	6	42.3	
A. A.	Coronary arteriosclerosis	17.6	1.26	0.222	6	55.3	

TABLE 2
The solids and potassium content of right ventricle

Subject	Chief diagnosis	Solids	Potassium in dry tissue	Potassium in wet tissue	Daily dose of K_2HPO_4	Duration of administration of potassium	Remarks
		per cent	per cent	per cent	grams	weeks	
E. A.	Lymphosarcoma	18.6	1.40	0.262	0	0	No cardiac disease
R. D.	Brain tumor	20.5	1.30	0.267	0	0	
J. S.	Carcinoma of esophagus	21.0	1.21	0.254	0	0	
J. F.	Syphilitic myocarditis	19.6	1.10	0.216	0	0	Cardiac failure; never received potassium dibasic phosphate
J. J.	Syphilitic aortic insufficiency	18.7	0.95	0.177	0	0	
J. B.	Chronic nephritic hypertension	18.5	0.94	0.172	0	0	
E. R.	Cardiac hypertrophy	17.5	0.97	0.170	0	0	
F. H.	Hypertension	18.6	0.95	0.177	0	0	
J. R.	Hypertension	19.9	0.85	0.148	0	0	
E. H.	Mitral stenosis	18.3	1.13	0.206	0	0	
G. H.	Hypertension	17.6	0.95	0.168	12	2.5	Cardiac failure; received potassium dibasic phosphate
H. M.	Mitral stenosis	19.2	1.12	0.215	6	6.3	
F. D.	Syphilitic aortic insufficiency	17.6	1.00	0.176	6	22.0	
B. P.	Hypertension	20.3	1.12	0.228	6	28.7	
A. D.	Hypertension	18.6	1.03	0.192	6	42.3	
A. A.	Coronary arteriosclerosis	17.5	0.92	0.161	6	55.3	

TABLE 3
The solids and potassium content of left ventricle

Subject	Chief diagnosis	Solids	Potassium in dry tissue	Potassium in wet tissue	Daily dose of K_2HPO_4	Duration of administration of potassium	Remarks
		per cent	per cent	per cent	grams	weeks	
E. A.	Lymphosarcoma	22.4	1.29	0.289	0	0	No cardiac disease
R. D.	Brain tumor	23.0	1.19	0.274	0	0	
J. S.	Carcinoma of esophagus	19.9	1.47	0.292	0	0	
J. F.	Syphilitic myocarditis	19.5	1.02	0.200	0	0	Cardiac failure; never received potassium dibasic phosphate
J. J.	Syphilitic aortic insufficiency	20.4	0.98	0.199	0	0	
J. B.	Chronic nephritic hypertension	19.9	1.02	0.203	0	0	
E. R.	Cardiac hypertrophy	18.6	1.07	0.199	0	0	
F. II.	Hypertension	19.4	0.83	0.162	0	0	
J. R.	Hypertension	19.9	1.10	0.221	0	0	
E. II.	Mitral stenosis	17.6	1.02	0.180	0	0	
G. II.	Hypertension	19.9	1.05	0.208	12	2.5	Cardiac failure; received potassium dibasic phosphate
J. C.	Syphilitic aortic insufficiency	19.2	1.12	0.215	6	5.0	
H. M.	Mitral stenosis	21.6	1.05	0.227	6	6.3	
F. D.	Syphilitic aortic insufficiency	19.3	1.16	0.224	6	22.0	
B. P.	Hypertension	22.0	1.24	0.274	6	28.7	
A. D.	Hypertension	18.4	1.12	0.206	6	42.3	
A. A.	Coronary arteriosclerosis	18.4	0.96	0.177	6	55.3	

TABLE 4
The solids and potassium content of liver

Subject	Chief diagnosis	Solids	Potassium in dry tissue		Potassium in wet tissue	Daily dose of K_2HPO_4	Duration of administration of potassium	Remarks
		per cent	per cent	per cent	grams		weeks	
E. A.	Lymphosarcoma	20.3	1.20	0.244	0	0		No cardiac disease
J. D.	Carcinoma of gallbladder	23.1	1.07	0.248	0	0		
J. F.	Syphilitic myocarditis	25.2	0.69	0.174	0	0		Cardiac failure; never received potassium dibasic phosphate
J. J.	Syphilitic aortic insufficiency	20.7	0.72	0.148	0	0		
J. B.	Chronic nephritic hypertension	21.5	0.80	0.173	0	0		
E. R.	Cardiac hypertrophy	18.1	1.11	0.201	0	0		
G. H.	Hypertension	20.5	0.77	0.158	12	2.5		
J. C.	Syphilitic aortic insufficiency	22.2	0.88	0.196	6	5.0		Cardiac failure; received potassium dibasic phosphate
H. M.	Mitral stenosis	21.7	0.72	0.155	6	6.3		
F. D.	Syphilitic aortic insufficiency	22.2	0.86	0.191	6	22.0		
B. P.	Hypertension	25.0	1.18	0.296	6	28.7		
A. D.	Hypertension	23.1	1.06	0.244	6	42.3		
A. A.	Coronary arteriosclerosis	23.0	1.27	0.291	6	55.3		

TABLE 5
The solids and potassium content of the kidney

Subject	Chief diagnosis	Solids	Potassium in dry tissue	Potassium in wet tissue	Daily dose of K_2HPO_4	Duration of administration of potassium	Remarks
		per cent	per cent	per cent	grams	weeks	
W. S.	Acute peritonitis	19.3	1.13	0.215	0	0	No cardiac disease
E. A.	Lymphosarcoma	16.6	1.76	0.292	0	0	
E. H.	Eclampsia	17.5	1.20	0.208	0	0	
J. D.	Carcinoma of gallbladder	20.4	0.83	0.170	0	0	
J. F.	Syphilitic myocarditis	16.6	0.68	0.114	0	0	Cardiac failure; received no potassium dibasic phosphate
J. J.	Syphilitic aortic insufficiency	17.8	1.00	0.178	0	0	
J. B.	Chronic nephritic hypertension	17.0	0.91	0.154	0	0	
E. R.	Cardiac hypertrophy	14.2	0.88	0.125	0	0	
G. H.	Hypertension	15.4	0.87	0.135	12	2.5	Cardiac failure; received potassium dibasic phosphate
J. C.	Syphilitic aortic insufficiency	17.2	0.99	0.170	6	5.0	
H. M.	Mitral stenosis	18.2	0.89	0.163	6	6.3	
F. D.	Syphilitic aortic insufficiency	15.3	0.91	0.144	6	22.0	
B. P.	Hypertension	18.7	1.13	0.212	6	28.7	
A. D.	Hypertension	16.1	1.09	0.176	6	42.3	
A. A.	Coronary arteriosclerosis	13.3	0.97	0.129	6	55.3	

TABLE 6
Average values for the solids and potassium content

Tissue	Solids			Potassium in dry tissue			Potassium in wet tissue		
	Subjects without cardiac disease	Subjects with cardiac failure, not receiving K_2HPO_4	Subjects with cardiac failure, receiving K_2HPO_4	Subjects without cardiac fatigue	Subjects with cardiac disease not receiving K_2HPO_4	Subjects with cardiac disease receiving K_2HPO_4	Subjects without cardiac disease	Subjects with cardiac disease not receiving K_2HPO_4	Subjects with cardiac disease receiving K_2HPO_4
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Skeletal muscle.....	24.8	20.3	22.4	1.35	0.98	1.26	0.335	0.199	0.297
Right ventricle.....	20.0	18.8	18.5	1.30	0.98	1.02	0.261	0.181	0.190
Left ventricle.....	21.8	19.3	19.8	1.32	1.01	1.10	0.285	0.195	0.219
Liver.....	21.7	21.4	22.5	1.13	0.83	0.96	0.246	0.174	0.219
Kidney.....	18.4	16.4	16.3	1.23	0.87	0.96	0.221	0.143	0.161

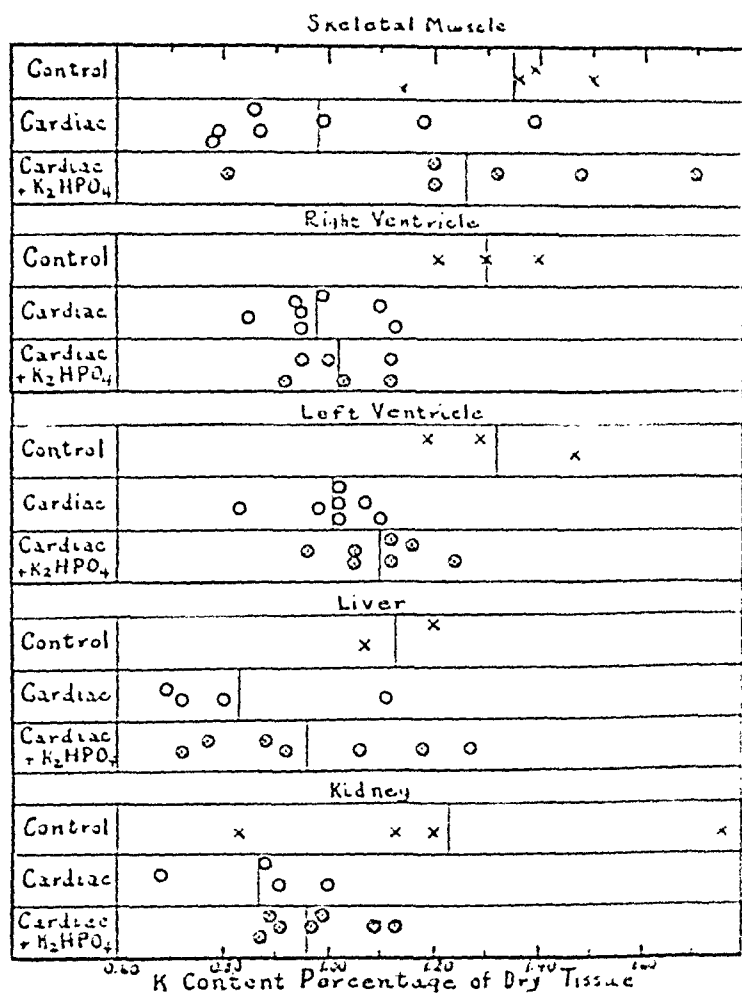


FIG. 1. POTASSIUM CONTENT IN DRY TISSUE
Vertical lines indicate average value for each type of tissue

DISCUSSION

Skeletal Muscle

Total solids. In the control subjects the percentage of total solids varied between 22.2 and 27.1 per cent with an average of 24.8 per cent. Most of the subjects with cardiac disease had lower solids, although three of them had values within, and one had a value above the control range. The average values for solids were higher in the subjects who did than in those who did not receive potassium phosphate but were below the controls in both groups. Two of the subjects (J. R. and M. H.) had had marked edema previously but at the time of death had no "pitting." The water content of their muscles was definitely high, low total solids indicating that moderate edema of the muscles may be present without demonstrable signs. The reverse situation, i.e., a high percentage of total solids in the muscle with "pitting" of the subcutaneous tissues, did not occur in this series. It seems that the presence of "pitting" may usually be interpreted as meaning that the regional muscles as well as the subcutaneous tissues are edematous, but the absence of pitting does not mean that the muscles of the legs are not edematous. When diuresis occurs and all evidence of edema disappears the muscles may (as in J. R. and M. H.) still be rich in water, or (as in F. H. and B. P. both of whom had been edematous in the past) their water content may diminish to normal.

Potassium. Inspection of figure 1 shows definitely that the potassium content of the skeletal muscle of patients with cardiac failure tends to run much lower than in the control. As is to be expected, the range is greater, and in the present series two of the seven cases fall within the control range. Administration of K_2HPO_4 , with one exception, restored the potassium content of skeletal muscle to the level of the controls. That this is a restoration of potassium seems most probable since the clinical history, treatment other than K_2HPO_4 administration, and the autopsy findings were similar in the two groups.

Cardiac Muscle

Total solids. The ventricles of the control subjects contained, as an average, about ten per cent more total solids than did those of the patients with cardiac disease. The content of solids in the patients

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